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Electrophoretic Assessment of the Contribution of Transplanted Scottish Atlantic Salmon (*Salmo salar*) to the Esva River (Northern Spain)

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Morán, P., A.M. Pendás, E. García-Vázquez, J.T. Izquierdo, and D.T. Rutherford. 1994. Electrophoretic assessment of the contribution of transplanted Scottish Atlantic salmon (*Salmo salar*) to the Esva River (Northern Spain). Can. J. Fish. Aquat. Sci. 51: 248–252.

The genetic influence of stocking on the Atlantic salmon (*Salmo salar*) population from the Spanish Esva River was analysed by electrophoresis of protein loci. Genetic variation within parr and mature male parr samples demonstrated that Scottish-origin parr stocked in 1990 survived the first summer. However, samples collected from returning adults in 1990 and 1991 did not indicate any contribution from previous stocking programs. Altered *MEP-2** frequencies indicate that the native gene pool may have been disrupted by foreign genomes from precocious males or grilse.

On a annalysé l'effet génétique des repeuplements de saumon Atlantique (*Salmo salar*) dans la rivière espagnole Esva par moyen de l'électrophorèse des loci protéiques. La variation génétique parmi les échantillons des tacons et des tacons mûrs a montré que les tacons écossais relâchés en 1990 ont survécu le premier été. Cependant, les échantillons des adults retournant en 1990 et 1991 n'ont pas montré aucune contribution des antérieurs programmes de repeuplement. Les fréquences modifiées de *MEP-2** indiquent que le pool génique indigène a été perturbé par des génomes étrangers de mâles précoces ou de tacons.

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Restocking programmes to supplement declining natural populations of Atlantic salmon (*Salmo salar*) have been applied in Northern Spain rivers during the last 20 yr (García de Leániz et al. 1987, 1989; Martín Ventura 1988). In the Asturian River Esva, multi-sea-winter (MSW) salmon return from March to late autumn and remain in freshwater until spawning (December–January); grilse generally return later from June to autumn. Salmon are exploited by rod and line from March to July, the average catch for recent years being 308 individuals in which the average percentage of grilse was 19.1% (Table 1). The exploitation rate of this population was estimated to be between 30 and 40%. Hatchery-reared parr, imported at the eyed-egg stage from different countries (Table 1), have annually been stocked into the Esva River (except in 1991). Chromosomal studies of native and the foreign donor stock indicated, however, that stocking success with Scottish salmon, at least in 1989, has been poor (García-Vázquez et al. 1991). García de Leániz et al. (1989) have shown the scarce contribution of salmon stocked from a Scottish hatchery to the angling fisheries in Cantabria (Northern Spain).

Transplanted fish generally have lower survival rates and disrupted homing ability compared with indigenous salmonids (Ritter 1975; Standal and Gjerde 1987; Reisenbichler 1988; García de Leániz et al. 1989). Once stocking has occurred, however, foreign genes could have been introduced into the genetic pool of the endemic populations with consequent risks of reduced fitness in the river environment (Saunders 1981; Rohovec et al. 1988). In stocking programs, gene flow between foreign stocked and native populations involves three life stages: (1) foreign juveniles survive to emigrate as smolts, (2) foreign male parr may reach precocious maturity, and (3) foreign individuals are able to return (as grilses or MSW), intermate, and spawn with the native salmon. The aim of this paper is to assess the possible gene flow between stocked fish and wild Esva River salmon. Electrophoretic analyses were used to investigate each of these stages.

Materials and Methods

In June 1990 the Esva River was stocked in the adjacent areas Agüera, Coto Chanona, and Orio, 5 km downstream, with parr from the River Shin (Scotland). The Esva River was not stocked in 1991. The *S. salar* population from the River Shin was genetically analyzed by Youngson et al. (1989). Parr samples of the Esva River were taken by electrofishing before and after stocking in Agüera and Coto Chanona. Muscle samples of adults were provided by anglers. The samples analyzed were as follows: *Native-90*: muscle and liver of parr 0+ captured in Coto Chanona, in May 1990 (before stocking); *Native-91*: muscle taken by biopsy of parr 1+ captured in Coto Chanona before sea migration, in February and March 1992; they were tested for maturity

TABLE 1. Number of salmon caught by anglers in the Esva River from 1986 to 1991 (N); proportion of grilse, number of foreign individuals released, and origin of foreign stock.

Year	N	Grilse (%)	Stocked	Origin
1986	806	11.80	25 000	Ireland
1987	400	10.80	100 000	Ireland
1988	171	20.50	25 000	Ireland
			100 000	Scotland
1989	141	31.20	52 500	Scotland
1990	127	26.00	60 000	Scotland
1991	203	14.30		

and then classified as immature or mature (i.e., precocious) parr; *Mixed-1*: muscle and liver of part 0+ captured four months after stocking, in September 1990, in Coto Chanona; *Mixed-2*: muscle and liver of parr 0+ captured 5 mo after stocking, in October 1990, in Agüera; *Prec-90*: muscle and liver of precocious parr 0+ (mature males) captured in October 1990 along the river, including the three stocked areas (sample size was small because the sampling time was too early for parr to reach maturity); *Adults*: muscle samples were from 84.3% of adults caught by anglers in 1990 and from 90.6% in 1991. Age was determined in adults by scale analysis; samples were classified as MSW (≥2SW) or grilse (1SW). Adult samples were supplemented with 30 adults (21 grilse and nine MSW) electrofished in 1990 and 17 adults (two grilse and 15 MSW) electrofished and 34 (eight grilse and 26 MSW) kelts in 1991. All samples were frozen at −20°C until electrophoresis analysis.

The structural protein loci analysed were selected for screening on the basis of their polymorphism in other *S. salar* populations (Ståhl 1987). The following enzymes and corresponding loci were analysed: creatine kinase (EC 2.7.3.2; *CK-1*, CK-2**), glycerol-3-phosphate dehydrogenase (EC 1.1.1.8; *G3PDH-1**), glucose-6-phosphate dehydrogenase (EC 5.3.1.9; *GPI-1,2*, GPI-3**), malate dehydrogenase (EC 1.1.1.37; *MDH-1*, MDH-2*, MDH-3,4**), malic enzyme (NADP+) (EC 1.1.1.40; *MEP-1*, MEP-2*, MEP-3**), and phosphoglucomutase (EC 5.4.2.2; *PGM-1*, PGM-2**) in muscle and aspartate aminotransferase (EC 2.6.1.1; *AAT-1,2*, AAT-3**), alcohol dehydrogenase (EC 1.1.1.1; *ADH**), isocitrate dehydrogenase (EC 1.1.1.42; *IDHP-2*, IDHP-3**), lactate dehydrogenase (EC 1.1.1.27; LDH-4*), and superoxide dismutase (EC 1.15.1.1; *SOD**) in liver. The iditiol dehydrogenase (EC 1.1.1.14; *IDDP-1*, IDDP-2**) was variable but zymogram interpretation was too unclear to provide us any guarantee. We used starch gel electrophoresis following the methods explained by Verspoor and Cole (1989). Nonemclature of genetic protein variation follows Shaklee et al. (1990).

G-tests were used for deviations of genotype frequencies from Castle–Hardy–Weinberg (CHW) equilibrium, with Williams correction for sample size when needed (Sokal and Rohlf 1981), and χ^2 tests for allelic heterogeneity (Workman and Niswander 1970), F_{is} as 1 − observed/expected heterozygotes (Wright 1931).

Contributions of source populations to the mixed samples were estimated using the maximum likelihood method of Fournier et al. (1984) and following the procedures outlined by Wood et al. (1987, 1989). Maximum likelihood point estimates are reported and standard deviations calculated from 100 bootstrap estimates of the mixture composition. Data for the Scottish (Shin) and Irish stocks were taken from published data. Data for Shin parr (transplanted in 1988, 1989, and 1990) are from Youngson et al. (1989), assuming that frequencies did not change over the few years. Irish parr were transplanted in 1986, 1987, and 1988 but their stock origins are unknown. Irish data are from McElligott and Cross (1991) and are represented by average values over 17 wild Irish populations.

Results

Genetic Differences between Native and Stocked Parr

Parr in the Native-90 sample were variable at six loci and differed from the Shin and Irish data at three alleles (*MEP-2*100, AAT-3*25*, and *IDH-4*100*; Table 2). Mean heterozygosity was also lower in the Native-90 sample compared with those estimated for the Shin and Irish data. All loci sampled in the Native-90 sample, and reported for the Shin and Irish data, were in CHW equilibrium.

Contribution of Stocked Parr to the Mixed Samples

Scottish parr were estimated to contribute 87 and 72% of the parr in samples Mixed-1 and Mixed-2, respectively (Table 3). These values were determined assuming that the Irish transplants had not contributed to the gene pools for the 1990 samples. The elevated frequency of *MDH-3,4*80* in Mixed-2 is problematic, however, since it has a low frequency in Native-90 and Shin samples. If the Irish stock is used in the mixture analysis of Mixed-2, then 98% of the sample is assigned to the Irish stock and no native fish are estimated to be present in this sample. However, *MDH-3,4** is not in CHW equilibrium in Mixed-2 ($G = 6.80$, $p < 0.01$) and this result could be a sampling artifact, since the *MDH-3,4*80* allele was present in the Native-90 sample.

Contribution of Stocked Parr to Precocious Males

While the sample size is small, the Shin stock was estimated to contribute 35% of the Prec-90 sample (Table 3). This result largely reflects the intermediate value of *MEP-2*100* in the Prec-90 sample compared with the Native-90 and Shin samples. However, to conclude that this result reflects true stock contributions assumes that malic enzyme does not influence precocious maturity. To examine this assumption the Native-91 samples were classified as immature or mature (precocious). Mature and immature parr did not differ at the *MEP-2*100* allele (Table 4), supporting the previous assumption.

Analysis of Adults

Analyses of the adults returning in 1990 and 1991 (Table 5) indicate that *MEP-2** allele frequencies differed between years ($\chi^2 = 35.201$, 6 df, $P < 0.001$), grilse (ages 2.1 and 1.1) have lower *MEP-2*100* frequencies than MSW adults returning in the same year, *MDH-3,4** allele frequencies were not significantly different between samples ($\chi^2 = 2.788$, 6 df, $p = 0.835$). In 1990 and 1991, each age class was in CHW equilibrium for both loci. One MSW salmon, caught in 1990, showed variation at the *PGM-2** locus.

Comparison of allele frequencies in adults returning from the 1988 brood year (age 1.1 grilse in 1990 versus age

TABLE 2. Allele frequency data in all the samples analysed (N = number of individuals analysed). Statistical significance of G-test for deviation from the CHW equilibrium. H = mean heterozygosity per locus. *$p < 0.05$; ** $p < 0.01$; ns, not significant.

Locus	Allele	Native-90 ($N = 64$)	Mixed-1 ($N = 42$)	Mixed-2 ($N = 42$)	Prec-90 ($N = 17$)	Shin ($N = 50$)	Ireland ($N = 40$)
*MEP-2**	*100*	0.92	0.48	0.49	0.68	0.44	0.53
	125	0.08	0.52	0.051	0.32	0.56	0.47
χ^2		ns	ns	ns	ns	ns	ns
*MDH-3,4**	*100*	0.99	1	0.87	0.94	1	0.93
	80	0.01	0	0.13	0.06	0	0.07
χ^2		ns		**	ns		ns
*PGM-2**	*100*	0.98	1	1	1	—	1
	80	0.02	0	0	0	—	0
χ^2		ns					
*GPI-3**	*100*	0.99	1	1	1	—	1
	90	0.001	0	0	0	—	0
χ^2		ns					
*AAT-3**	*100*	0.75	0.83	0.76	0.74	0.74	0.75
	50	0.25	0.16	0.24	0.26	0.16	0.25[a]
	25	0.00	0.01	0.00	0.00	0.10	—
χ^2		ns	ns	ns	*	ns	ns
*IDHP-4**	*100*	0.99	1	0.99	0.97	0.97	0.88
	116	0.01	0	0.01	0.03	0.03	0.12
χ^2		ns	ns	ns	ns	ns	ns
H		0.101	0.390	0.279	0.249	0.229	0.298

[a]*AAT-3*50* and *AAT-3*25* pooled.

TABLE 3. Estimated stock composition of mixture samples (standard deviations of the estimates in parentheses).

Sample	N	Proportion allocated to: Native	Shim
Mixed-1	42	0.134 (0.132)	0.866 (0.132)
Mixed-2	42	0.279 (0.148)	0.721 (0.149)
Prec-90	17	0.651 (0.175)	0.349 (0.175)

1.2 MSW in 1991) showed significant difference only for *MEP-2*100* ($\chi^2 = 20.93$, 2 df, $p < 0.001$). Higher frequency of heterozygotes in the grilse sample is consistent with Jordan et al. (1991). Mixture analyses of these adults support this interpretation, since the maximum likelihood estimates of stock origins (1.0 ± 0.085 native, 0.0 ± 0.011 Shin, 0.0 ± 0.086 Irish) for salmon from this broodyear do not indicate return of any foreign stock.

Stability of Gene Frequency between Generations

Gene frequencies varied significantly in three of four comparisons possible between generations. Native-90 and Native-91 samples differed in *MDH-3,4*100* frequency ($\chi^2 = 7.57$, $P = 0.006$) but not for *MEP-2** ($\chi^2 = 3.29$, $p = 0.0694$). Significant differences in *MEP-2*100* and *MDH-3,4*100* frequencies ($\chi^2 = 4.09$, $P = 0.04$; $\chi^2 = 5.45$, $p = 0.02$, respectively) existed between the Native-91 sample and parental frequencies (Grilse-90 + MSW-90).

Discussion

Atlantic salmon (Shin stock) transplanted to the Esva River, Spain, in 1990 survived through the first summer period and some matured precocially in the fall. These results do not support a previous assessment of transplanted Scottish parr (García-Vázquez et al. 1991). García-Vázquez et al. (1991) determined, using chromosome patterns, that Scottish parr failed in the Esva River in 1989, probably due to drought conditions that summer. However, in age 1.1 (1990 grilse) and 1.2 adults (1991 MSW), there was no evidence of contributions from Irish or Scottish stock transplanted in the 1988 broodyear. To date, therefore, there is evidence of foreign stock surviving in freshwater but no evidence of adult returns and spawning.

The higher frequency of *MEP-2*100/125* individuals in the 1990 grilse (age 1.1) than in the 1991 MSW (age 1.2) does support previous work by Jordan et al. (1991). If *MEP-2** heterozygotes do have a greater propensity to return as grilse, then the Scottish and Irish transplants should return mostly as grilse. A consequence of stocking could then be an increase in the proportion of Esva River salmon returning as grilse. It is notable that following the failure of Scottish transplants in 1989, there was actually a considerable decrease of the proportion of grilse in 1991. The significant difference in *MDH-3,4** allele frequencies between the Native-90 and Native-91 samples could indicate disruption of the native gene pool by foreign stock. Significant differences were also identified between the 1990 adult samples and the Native-91 sample, with lower frequency of the **100* alleles in the Native-91 sample. The latter would be consistent with a greater contribution to the next generation from grilse adults than from MSW salmon. However, this could simply reflect the relative numbers of grilse in the spawning population, since grilse return later than MSW salmon and mostly after angling season. Alternatively, in the 1990 broodyear, gene flow consistent with this observed difference could also have resulted from precocious males of Scottish origin. Precocious male parr can contribute as parents and fertilize proportions of eggs per redd (Hutchings and Myers 1988). The Scottish (Shin) stock does have lower frequency of the **100* allele, at both the *MEP-2** and *MDH-3,4** loci, than the native samples.

TABLE 4. Frequencies of *MEP-2** and *MDH-3,4** alleles in a sample of native parr aged 1+ taken in the Esva River in January–February 1992; χ^2 values and statistical probability p = testing difference between frequencies of precocious and immature. N = number of parr.

Allele	Whole (N = 243)	Precocious (N = 34)	Immature (N = 209)	χ^2	p
*MEP-2*100*	0.862	0.882	0.859	0.272	0.602
*MDH-3,4*100*	0.928	0.941	0.926	0.206	0.65
Total				0.478	0.787

TABLE 5. Number of adults observed of each genotype during the 1990 and 1991 angling season. P = allelic frequencies of the **100* allele; P is pooled by sea age (grilse and MSW).

Sampling year	Age	*MEP-2**			P	*MDH-3,4**			P
		100/100	*100/125*	*125/125*		*100/100*	*100/80*	*80/80*	
1990	2.2	2	0	0	0.962	2	0	0	0.97
	1.2	59	5	0		60	4	0	
	2.1	4	0	0	0.849	4	0	0	0.965
	1.1	36	15	1		48	4	0	
Total		101	20	1	0.91	114	8	0	0.968
1991	2.2	41	2	0	0.968	38	5	0	0.953
	1.2	145	9	1		141	14	0	
	2.1	3	0	0	0.917	3	0	0	0.97
	1.1	27	5	0		30	2	0	
Total		216	16	1	0.962	212	21	0	0.955

We are unable to differentiate these three alternative explanations (gene flow via precocious males and/or grilse adults, and/or increased numbers of grilse through fishing effects) with the information available. Greater contrast in genetic markers (more loci, greater stock specificity, etc.) between the transplanted and native stock will be required to test these hypotheses. In order to learn from future transplant "experiments", these information needs should be considered in selecting donor stocks, particularly if the management objectives remain to increase catch of MSW salmon while minimizing disruption of the natural genetic pool of the Esva River salmon.

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Thermal Niche of Larval Sea Lamprey, *Petromyzon marinus*

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Holmes, J.A., and P. Lin. 1994. Thermal niche of larval sea lamprey, *Petromyzon marinus*. Can. J. Fish. Aquat. Sci. 51: 253–262.

We used behavioral and physiological responses of larval sea lamprey, *Petromyzon marinus*, to temperature to describe the thermal niche and define optimal thermal habitat. Acclimation temperature had a weak direct positive effect on acute preferred temperatures in summer but no effect on acute preferred temperatures in winter tests. Swimming speed, mass, and acclimation temperature accounted for 66% of the variation in the active metabolic rate of larval sea lamprey. When larvae are swimming at their maximum prolonged rate, the relationship between temperature and metabolic rate is a unimodal curve, as has been found for more active fish species. The final preferred temperature in summer, 20.8°C, is similar to the temperature at which maximum scope for activity occurs, approximately 19°C. We propose the "fundamental thermal niche" of larval sea lamprey to be the range of temperatures between 17.8 and 21.8°C. Some practical applications for the thermal niche are sketched with respect to the integrated management of sea lamprey in the Great Lakes.

Nous avons utilisé les réactions comportementales et physiologiques des larves de grande lamproie marine, *Petromyzon marinus*, à la température pour décrire la niche thermique et définir l'habitat thermique optimal de cette espèce. La température d'acclimatement avait un effet direct faiblement positif sur les températures aiguës préférées en été mais aucun effet sur les températures aiguës préférées dans les tests réalisés en hiver. La vitesse de nage, la masse et la température d'acclimatement expliquaient 66 % de la variation du métabolisme actif des larves de lamproie. Lorsque les larves nagent à leur vitesse maximum soutenue, la relation entre la température et le métabolisme est une courbe unimodale, comme on l'a observé chez des espèces de poisson plus actives. La température finale préférée en été, 20,8 °C, est similaire à celle pour laquelle on observe la fourchette maximale d'activité, soit environ 19 °C. Nous considérons que la « niche thermique fondamentale » des larves de grande lamproie marine est la fourchette de température comprise entre 17,8 et 21,8 °C. Nous présentons brièvement certaines applications pratiques de cette notion de niche thermique en vue de la gestion intégrée de la grande lamproie marine dans les Grands Lacs.

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Fish respond to temperature in their environment in a manner similar to their response to ecological resources such as food (Magnuson et al. 1979). This response guides fish to temperatures at which their physiological functions are relatively efficient, allowing them to compete and cooperate effectively with other species (Fry 1947; Brett 1971; Beitinger and Fitzpatrick 1979). Fish tend to select habitat temperatures within a "fundamental thermal niche", defined as ±2°C of the final preferred temperature measured in a laboratory gradient (Magnuson et al. 1979) when other factors do not limit access to those waters. Habitat temperatures within the range of a species' thermal niche influence its survival, growth, reproductive success (Shuter et al. 1980; Crowder and Magnuson 1982), and abundance (Christie and Regier 1988).

Relatively little quantitative information is available concerning the thermal ecology of larval sea lamprey, *Petromyzon marinus*, particularly as it influences habitat selection, abundance, and life history parameters such as growth rate. Habitat partitioning among lamprey species in streams appears to occur along temperature gradients (Morman 1979), suggesting that each species selects different temperatures. The preferred temperature of larval sea lamprey in the winter is approximately 13.6°C (Reynolds and Casterlin 1978). Empirical studies show that larval sea lamprey grow faster in streams with moderately warm temperatures than in streams with cold temperatures (Holmes 1990; Young et al. 1990) and metamorphose to the parasitic stage at a younger age in warmer waters (Purvis 1980).

The objectives of this study are to describe the summer thermal niche of larval sea lamprey and to assess the utility of a compound Arrhenius model for describing the physiological responses of larval sea lamprey to temperature. The thermal niche, which encompasses both behavioral and physiological responses to temperature, is estimated from experiments on the behavioral thermoregulation and active metabolism of larvae through their temperature tolerance range. Because it is impractical to measure the thermal optima of many activities, we assume that the temperature at which maximum scope for activity occurs corresponds to the temperature at which growth rate is maximal, as has been found for other species (Beitinger and Fitzpatrick

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1979). We also illustrate measures of optimal thermal habitat in streams based on the thermal niche.

There are few studies of the physiological or behavioral responses of larval lamprey to temperature. For this reason and because the larvae differ from teleost fishes, both morphologically and behaviorally (larval lamprey are blind, filter-feeding, sedentary animals living in burrows in the sediments of streams), our analyses were used to describe the patterns of change in the data rather than to test specific a priori hypotheses. We then attempt to check the veracity of our findings with respect to other fishes.

Biologists have used a variety of mathematical expressions to describe the thermal responses of ectothermic organisms (Fry 1947; Cossins and Bowler 1987). In this paper, we use two versions of the Arrhenius model to describe the standard and active metabolism of larval sea lamprey. We prefer these models over others because they appear to have a stronger theoretical basis (Cossins and Bowler 1987; Regier et al. 1990).

Materials and Methods

Preferred Temperature

Larval sea lamprey (n = 500) were collected in July and October 1988 from Salem Creek, Lake Ontario (44°00′N, 77°50′W), for the thermoregulation experiments. The larvae collected in July were held at ambient conditions (20°C and a 14 h light : 10 h dark photoperiod) until late August 1988 when acclimation to temperatures of 7, 10, 15, 20, and 25°C (±1°C) began. Water temperatures were changed by 1°C·d^{-1} until the desired acclimation temperature was reached, and the larvae were then held at that temperature for at least 4 wk prior to testing. Acclimation temperatures and photoperiod were held constant until the summer of 1989 when the first preference tests were performed and were then cycled to mimic natural conditions at Toronto (43°45′N, 79°20′W). Preferred temperatures were also determined during the winter (8.5 h light:15.5 h dark photoperiod) for comparison with a previous winter study (Reynolds and Casterlin 1978).

Larvae for the thermoregulation experiments were randomly distributed into five circular plastic 35-L acclimation tanks (20·tank^{-1}), with total masses ranging from 27.0 to 27.6 g·tank^{-1} initially. Initial lengths and weights did not differ significantly ($p > 0.05$) among the tanks. Sand substrate (7 cm) was provided in trays to minimize temperature gradients within the sand and between the sand and water. The acclimation tanks were supplied with aerated dechlorinated tap water at rates which resulted in turnover times of 2.5–3 d. Temperatures were regulated with thermostatically controlled 50-W aquarium heaters in each tank. Larvae were fed 15 g dry brewers yeast·tank^{-1}·wk^{-1}. The tanks and the top 2–3 cm of sand were cleaned to remove yeast, metabolic wastes, and dead larvae every 2 wk. All of the sediment in the 20 and 25°C tanks was replaced at bimonthly intervals. Dead larvae were replaced using larvae held at 14°C in aquariums; these animals may have been held for significantly shorter periods at the same temperature. Mortality in the 12 mo prior to the summer acclimation period consisted of 4, 5, and 15 larvae in the 15, 20, and 25°C tanks, respectively. During the 5 mo prior to the winter acclimation period, 4, 2, 10, 21, and 5 dead larvae were replaced in the 7, 10, 15, 20, and 25°C tanks, respectively. There was no mortality in any tank in the 4 wk prior to testing.

Acute preferred temperature was determined in a plastic trough 1.3 m long × 15 cm wide × 12.5 cm high divided into 10 test compartments (12 cm long × 15 cm wide × 10 cm high) by plastic partitions over which larvae had to swim to move between compartments. A temperature gradient of 23–30C° was established by regulating heating and cooling in two small end compartments (6 cm long) using two rheostatically controlled 200-W immersion heaters and continual replenishment of crushed ice in the water. Perforated 5-mm plastic tubing along the bottom of the trough provided aeration and minimized thermal stratification in the test compartments. Water temperature was measured at the bottom-centre of each compartment and the median of the measurements was used for statistical analyses. The difference between initial and final temperatures averaged (SD) 1.0 (0.9C°) in the summer tests and 1.3 (1.0C°) in the winter tests.

Larval sea lamprey were tested in groups of five to determine temperature preference because it was logistically impossible to test each larva individually. The larvae were placed in the gradient at the point corresponding to their acclimation temperature and 5 min was allowed for habituation to this environment. Individuals that moved to the extreme ends of the gradient during this time were removed, but were not replaced, because we inferred that they were not responding primarily to temperature in the test environment. Four individuals were removed during the summer test period (one at 20°C and three at 25°C) and 16 individuals were removed during the winter tests (four at 7°C, five at 10°C, three at 15°C, and four at 20°C). Trials were run for 30 min in a room with all lights off except for a red darkroom safelight. A fluorescent light was positioned 40 cm above the centre of the gradient and was turned on for 30 s every 3 min to stimulate movement and temperature selection (McCauley et al. 1978) and the position of each larva was recorded when the light was turned on. Each group of larvae was retested with the gradient reversed to eliminate orientation to nonthermal gradients. All tests were carried out between 09:30 and 21:30 in the summer and between 10:00 and 19:00 in the winter. The number of animals tested, mean sizes tested, and testing dates are listed in Table 1. Although larval length was smaller at the higher acclimation temperatures than at the lower temperatures, all larvae tested were from the oldest age-group (3+) in Salem Creek at the time of collection (Holmes 1992). Therefore, preferred temperature should not be confounded by age in our analysis.

Data from the two replicate runs of each test group were pooled and occurrences at temperatures ≥31.4°C, i.e., the lethal temperature as determined by Potter and Beamish (1975), were removed. Such occasions were artifacts of the recording method, since the larvae were moving when the lights were turned on, making it difficult to record their position in the gradient. Of 1850 position observations in the summer data, 38 (2.0%) were at temperatures >31.4°C compared with 60 (3.7%) of 1606 observations during the winter. These data sets were checked for normality using the Shapiro–Wilk W statistic (SAS Institute 1987) and the acute preferred temperature was estimated as the arithmetic mean temperature occupied during the two runs.

Least-squares regression was used to (1) assess the effect of acclimation temperature on preferred temperature and

TABLE 1. Summary of test dates and number and mean lengths (mm) of larval sea lamprey used to determine acute temperature preferences and active metabolism. S indicates summer period and W denotes winter. Sample sizes represent the number of larvae tested after removing those that did not adjust to the test environment.

Acclimation temperature (°C)		n[b]	n		
Target	Observed[a]	tested	removed	Mean TL (±SD)	Test dates
		Preferred temperature experiments			
7 S	6.4 (1.6)	19 (4)	0	107.6 (13.2)	8–9 Sept. 1989
W	6.2 (0.6)	16 (4)	4	101.5 (13.9)	12–19 Mar. 1990
10 S	9.6 (1.1)	19 (4)	0	106.7 (8.22)	6–7 Sept. 1989
W	10.4 (0.8)	15 (4)	5	102.5 (9.68)	6–8 Mar. 1990
15 S	15.3 (0.6)	20 (4)	0	96.4 (12.6)	21–23 Aug. 1989
W	15.5 (0.6)	15 (4)	3	92.0 (9.04)	28 Feb. – 2 Mar. 1990
20 S	20.0 (0.7)	16 (4)	1	88.4 (8.96)	28–31 Aug. 1989
W	19.1 (1.6)	13 (4)	4	84.8 (6.61)	21–22 Feb. 1990
25 S	24.6 (0.4)	10 (2)	3	85.7 (6.94)	5 Sept. 1989
W	23.2 (2.6)	15 (3)	0	85.4 (6.22)	20–21 Feb. 1990
		Active metabolism experiments			
7 S	7.1 (0.7)	5 (5)	0	104.6 (6.95)	4–14 July 1990
10 S	10.0 (0.6)	6 (6)	0	113.2 (8.84)	27 Aug. – 10 Sept. 1990
15 S	15.3 (0.5)	6 (6)	0	108.2 (9.32)	17–29 July 1990
20 S	20.1 (0.4)	5 (5)	0	107.6 (7.64)	31 July – 12 Aug. 1990
25 S	25.1 (0.3)	6 (6)	2	101.3 (11.9)	13–25 Aug. 1990

[a]Mean temperature (SD).
[b]Numbers in parentheses are the number of independent groups tested and represent the correct number of degrees of freedom in statistical tests (Mathur and Silver 1980).

(2) estimate the final preferendum. The temperature at which the regression line fitted to the acute preference data intersected the line of equality (acclimation temperature equals preferred temperature) was taken as the final preferendum for that season. The 95% CLs of the slopes of the lines were calculated so that our results could be compared with the literature. The data from each test group were weighted by the number of larvae actually tested, but each test group provides only one degree of freedom (Mathur and Silver 1980; see Table 1) because larvae were not tagged to follow individual movements.

Active Metabolism

Sea lamprey larvae were collected from Port Britain Creek, Lake Ontario (43°56′N, 78°23′W), in early June 1990 for the metabolism experiments. Larvae (>90 mm TL, $n = 78$) were held in a 200-L tank supplied with dechlorinated tap water (≃ 14°C) for about 2 wk prior to the experiments and fed 4.5 g yeast·individual^{-1}·wk^{-1} to ensure that starvation was not a factor. Ten larvae were acclimated to each temperature, with total masses ranging from 15.6 to 20.9 g·tank^{-1} (Table 1). Once the desired temperature was reached, larvae were held for 2 wk prior to testing. There was no larval mortality except in the 25°C tank (two dead larvae).

A modified 1.55-L Blazka respirometer was used to determine the metabolic rate of individual sea lamprey at different swimming speeds between 09:00 and 20:00 daily. Water temperature was maintained in the respirometer (±0.5°C of desired temperature) by manipulating air temperature in a small environmental room in which the measurements were made. Two methods were used to stimulate continuous swimming in larvae: (1) the rear screen was intermittently electrified with a 10-V AC, 500-mA charge and (2) a fluorescent light was placed 30 cm above the rear screen, as the larvae are negatively photokinetic (Reynolds and Casterlin 1979).

The protocol for larvae acclimated to 7, 10, 15, and 20°C consisted of a habituation period, a preliminary testing and conditioning period, and a period in which the metabolism measurements were taken. Each test larva was placed in the respirometer and allowed to habituate for 12–18 h. Preliminary testing was conducted to determine the approximate range of water velocities over which the larva would swim continuously. Each larva was forced to swim for 5 min at fixed velocities, which increased from 3 cm·s^{-1} in steps of 0.6–0.8 cm·s^{-1} until the larva fatigued. After a 1-h recovery period, each larva was conditioned to the water current for two 1-h periods at velocities between 7 and 12 cm·s^{-1}. Conditioning was implemented to reduce variability in behavior that would contribute to error in estimates of oxygen consumption (Brett 1964; Beamish 1970); we did not test the validity of this assumption. Larvae acclimated to 25°C were habituated for 12–18 h and then tested without a conditioning period, beginning at a velocity of 6 cm·s^{-1} and continuing for two 2 cm·s^{-1} increments in most tests.

Definitive measurements of metabolic rates were made by forcing each larva to swim for 1 h at fixed velocities, beginning at 6–7 cm·s^{-1} and increasing in steps of 1.2–2.5 cm·s^{-1} until a larva fatigued. The use of uneven velocity increments likely influences estimates of critical swimming speed, but was necessitated by equipment limitations (see Holmes 1992). A 1-h recovery period was allowed between velocity increments during which the

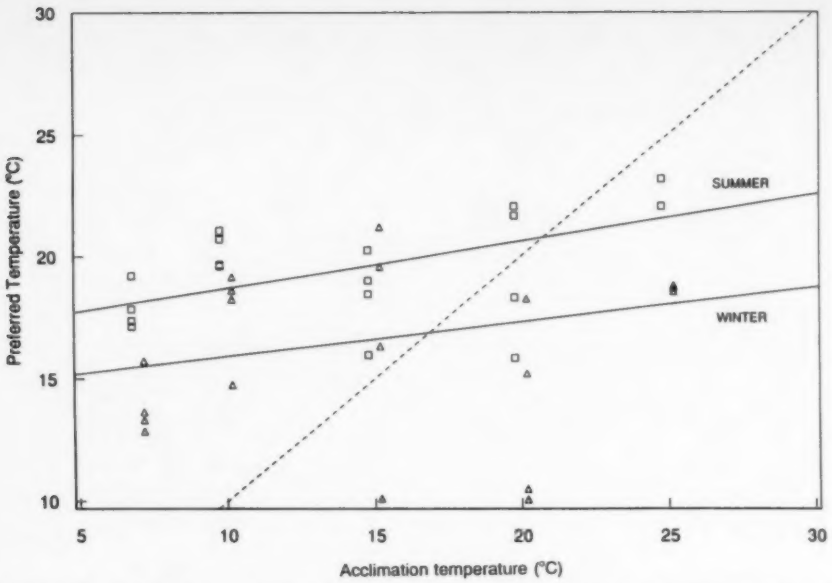

FIG. 1. Relationship between acute preferred temperature and acclimation temperature in larval sea lamprey acclimated to 7, 10, 15, 20, and 25°C in summer (squares) and winter (triangles). Data are weighted by the number of larvae tested in each group and are offset from the acclimation temperatures for illustration. The estimated final preferenda are the points at which the regression lines cross the line of equality (broken line).

respirometer was covered, flushed with water, and a current of 3.1 cm·s^{-1} was maintained to ensure thorough mixing of the water. When fatigued, a larva would not respond to electrical stimulation while being held by the water current against the rear screen. After fatigue occurred, the larva was removed from the respirometer, its total length and weight were measured, and a blank was run for 1 h to estimate microbial oxygen consumption.

Triplicate water samples were taken for initial and final dissolved oxygen determinations, the difference being the oxygen consumption of a larva. Dissolved oxygen was measured using a micro version (Burke 1962) of the azide modified Winkler technique (APHA 1981). The precision of this technique was ±0.2 mg O_2·L^{-1}. The sodium thiosulphate titrant was calibrated every 2 wk.

Oxygen consumption data from final runs were not used in our analysis if fatigue occurred within 20 min of the start of the last imposed velocity because of measurement error associated with dissolved oxygen determinations. In these instances, data from the highest completed velocity increment were used. Maximum prolonged swimming speed (Webb 1975) was corrected for times between 20 and 60 min on a proportional basis by calculating the "critical velocity" (Brett 1964):

$$(1) \quad V_C = V_L + \frac{T_F}{T_L}\Delta V$$

where V_C is the critical velocity, V_L is the last fully completed velocity, ΔV is the velocity increment, T_F is the time swimming prior to fatiguing, and T_L is the time period of completed runs (60 min). The effect of shocking on the oxygen consumption of a larva (milligrams of oxygen per hour) was removed by linear regression and these data were used to calculate the metabolic rate (milligrams of oxygen consumed per gram of larva per hour).

Arrhenius Models of Metabolism

Physiological and ecological rate processes of ectotherms generally exhibit two kinds of thermal response: (1) monotonic change between two extremes at which the function falls to zero and with a maximum near the upper extreme and (2) a curved or unimodal function (either symmetrical or asymmetrical about the mode) with the maximum away from the upper extreme and a gradual decline from the maximum to zero at the extremes. The rate of egg development and standard metabolic rate are usually of the first type, while growth rate and active metabolic rate are representative of the second type of response.

The "simple" Arrhenius model was first proposed by Arrhenius in 1898 to describe the effects of temperature on biochemical rate phenomena (Cossins and Bowler 1987). Regier et al. (1990) provided a rationale for its use with "monotonic" functions of the type described above for aquatic ectotherms. The influence of temperature on a particular rate constant, k, is

$$(2) \quad k = Ae^{-\frac{B}{T}}$$

where A and B are empirical constants and T is temperature (degrees Kelvin).

The "compound" Arrhenius model was derived by Johnson and Thornley (1984) to describe the temperature dependence of plant processes. Such rate processes are usually unimodal functions with optimum temperatures well within the usual temperature range of a particular species. The compound model is

TABLE 2. Total swimming time and prolonged swimming speed (critical velocity, V_C from equation 1 in text) of larval sea lamprey. Data are means (SE).

	Acclimation temperature (°C)				
	7	10	15	20	25
n	5	6	6	5	6
Time (h)	2.72 (0.23)	2.60 (0.21)	3.73 (0.31)	4.04 (0.28)	2.15 (0.04)
Critical velocity ($BL \cdot s^{-1}$)	0.97 (0.032)	1.02 (0.032)	1.31 (0.064)	1.43 (0.057)	0.86 (0.054)

TABLE 3. Parameter estimates and summary statistics for the compound Arrhenius model of sea lamprey active metabolism (see equation 6 in the text for explanation of the parameters).

Parameter	Estimate	SE
m	−0.656	0.258
α	1.508	0.206
A	63.358	31.086
B	18425.318	8748.726
C	105.766	16.300
D	30353.862	4460.614

Model statistics

Source	df	SS	MS
Regression	6	25.22	4.204
Residual	80	3.311	0.0414
Total	86	28.538	
Corrected total	85	9.624	
$R^2 = 0.66$			

$$(3)\quad k = A\frac{e^{-\frac{B}{T}}}{1+e^{C-\frac{D}{T}}}$$

where C and D are constants and T is temperature (degrees Kelvin). The thermodynamic meaning of all the parameters in equations 2 and 3 is discussed in Sharpe and DeMichele (1977) and Johnson and Thornley (1984).

By definition the total metabolic rate at a given temperature and swimming speed is the sum of standard and active metabolism. Standard metabolic rate (SMR) of aquatic ectotherms is a function of mass, W, and acclimation temperature (Robinson et al. 1983). A modified model derived from the simple Arrhenius relationship is

$$(4)\quad \mathrm{SMR} = AW^m e^{-\frac{B}{T}}$$

where m is a constant of about −0.2 (Robinson et al. 1983). Active metabolism is the metabolism of a mobile or working animal and is a function of the mass, swimming speed, and acclimation temperature (e.g., Brett 1964, 1971; Beamish 1970). When temperature is constant, the expression

$$(5)\quad \mathrm{AMR} = \mathrm{SMR}\, e^{\alpha V},$$

where α is a constant and V is swimming speed (centimetres per second), has been found to be an acceptable descriptor of the relationship between active metabolic rate (AMR) and swimming speed (Brett 1964; Webb 1975). As the swimming speed reaches the critical velocity (maximum prolonged speed), temperature becomes a limiting factor. Under these conditions, the influence of temperature may be described by the compound Arrhenius model as

$$(6)\quad \mathrm{AMR} = AW^m e^{\alpha V}\frac{e^{-\frac{B}{T}}}{1+e^{C-\frac{D}{T}}}.$$

We used nonlinear least-squares regression (SAS Institute 1987) to fit equation 6 to the sea lamprey data. This is an iterative procedure in which the ability to converge on a solution depends on the initial parameter values given to the model. Our initial starting values were m = −0.5, α = 1.45, A = 52, B = 15152, C = 86.4, and D = 24776, based on experience fitting the different versions of the Arrhenius model to data from other species and literature values (e.g., Robinson et al. 1983; Regier et al. 1990).

Results

Preferred Temperature

Average acute preferred temperatures were higher in summer than in winter for all acclimation temperatures (Fig. 1). Larvae acclimated to 20 and 25°C selected temperatures lower than or nearly equal to their acclimation temperature regardless of the season. The regression line fitted to the summer data had a significant ($F_{1,16} = 9.35$, $p = 0.0075$) positive slope, with 95% CLs of 0.059 and 0.33. The slope of the line fitted to the winter data was not significantly different from zero ($F_{1,17} = 1.72$, $p = 0.207$) and its 95% CLs were −0.086 and 0.37. The regressions fitted to the seasonal data sets are as follows:

$$(7)\quad \mathrm{PT}_S = 16.78 + 0.192\ \mathrm{AT}$$
$$r^2 = 0.36,\ N = 18$$

$$(8)\quad \mathrm{PT}_W = 14.49 + 0.142\ \mathrm{AT}$$
$$r^2 = 0.092,\ N = 19$$

where S and W refer to summer and winter, respectively, PT is acute preferred temperature, and AT is acclimation temperature. The absence of an acclimation effect in the winter is qualified by the power of the regression, which was 0.24 (two-tailed test of $r = 0.30$, $\alpha = 0.05$ in Cohen 1969). Hence, there was a 76% chance of incorrectly concluding that acute preferred temperatures were not significantly different (i.e., $r = 0$). Assuming the computed r (0.30) is true, then a total sample of 84 (17 per acclimation tem-

TABLE 4. Comparison of metabolism measurements of various lamprey ammocoetes over a range of temperatures. Metabolic data are mg $O_2 \cdot g^{-1} \cdot h^{-1}$.

Species	Temp. (°C)	Metabolic rate	Mean weight (g)	n	Remarks
Ichthyomyzon fossor	18	0.147	5.1	5	Routine metabolism; free to move (Leach 1946)
	18	0.144	4.2	5	
	18	0.153	3.8	5	
	18	0.154	3.1	5	
	18	0.168	2.7	5	
Petromyzon marinus	18	0.168	5.4	5	Routine metabolism; free to move (Leach 1946)
	18	0.158	4.6	5	
	18	0.177	3.8	5	
	18	0.203	3.0	5	
	18	0.217	2.6	5	
	22.5	0.543	3.4	5	
	22.5	0.326	3.5	5	Transforming ammocoetes
Lampetra japonica	2	0.0299	3.63	50	Routine metabolism in plastic strands (Bourque 1974)
	10	0.0296	3.92	47	
	18	0.0410	4.10	42	
Ichthyomyzon hubbsi	3.5	0.0116	1.27	10	Routine metabolism in glass beads (Hill and Potter 1970)
	9.5	0.0320	1.01	9	
	15.5	0.1380	0.14	33	
	15.5	0.0731	1.24	12	
	15.5	0.0554	3.44	12	
	22.5	0.1290	1.22	10	
Petromyzon marinus	7	0.038	1.92	5	Standard metabolism estimated by extrapolation to zero activity (this study)
	10	0.057	2.26	6	
	15	0.086	1.93	6	
	20	0.060	1.86	5	
	25	0.033	1.81	6	

perature) would be required to ensure a power of 0.80 or a sample size of 139 (28 per acclimation temperature) if a power of 0.95 (i.e., $\alpha = \beta = 0.05$) is desired.

Equations 7 and 8 represent the lines of best fit to the data in Fig. 1 and were used to estimate the final preferendum and the effect of season on the final preferendum, although the r^2 values indicate that neither relationship has high predictive power. The estimated final preferendum of sea lamprey larvae was 20.8°C in summer and 16.8°C in winter. The mean of the preferred temperatures in summer (19.7°C) was significantly greater ($t = 7.15$, $p < 0.05$) than the mean in winter (16.7°C). This may imply that the final preferenda in the two seasons also differ statistically.

Metabolic Rates

The swimming performance of larval sea lamprey in the respirometer followed a consistent trend (Table 2), increasing to a peak at 20°C, after which it declined sharply. Swimming performance at 25°C was poorer than at colder acclimation temperatures (7 and 10°C). Larvae acclimated to 15 and 20°C swam significantly longer and at higher speeds than larvae acclimated to 7, 10, and 25°C ($p < 0.05$). The maximum observed swimming speed was about 16 $cm \cdot s^{-1}$ for animals acclimated to 20°C. The measures of swimming performance used here are correlated; larvae that swim longer in the respirometer, i.e., through more velocity increments, must necessarily attain greater prolonged swimming speeds.

The model fitting procedure successfully converged on the parameter estimates shown in Table 3. This solution is relatively robust, as we changed the starting values and obtained equivalent solutions with the same error sums of squares. Swimming speed, mass, and temperature together accounted for 66% of the variation in the metabolic rate data, with swimming speed explaining the largest proportion of the variation (Holmes 1992). The sea lamprey data used here are not all independent, i.e., $n \neq 87$. Only 28 different animals were used and three to four data points were collected for each animal. Thus the degrees of freedom shown in Table 3 are inflated.

The plots of standard and active metabolic rates in Fig. 2 were obtained by substituting swimming speeds of 0 $cm \cdot s^{-1}$ (standard metabolism) and the maximum prolonged speeds in Table 2 after conversion to centimetres per second (active metabolic rate) into the model in Table 3. The curves are anchored by the inference that a larva is not capable of metabolic activity at temperatures below 0°C or above 31.4°C (the upper lethal level reported by Potter and Beamish 1975). The maximum scope for activity (the difference between standard and active metabolic rates) in these data occurs at a temperature of approximately 19°C. Maximum metabolic rates at 15 and 20°C were 0.67 and 0.59 mg $O_2 \cdot g^{-1} \cdot h^{-1}$, respectively, compared with rates of 0.23 and 0.14 mg $O_2 \cdot g^{-1} \cdot h^{-1}$ at 7 and 25°C, respectively.

Discussion

Larval sea lamprey can be placed in the coolwater thermal guild based on a summer preferendum of 20.8°C (this study) and an ultimate upper incipient lethal temperature of 31.4°C

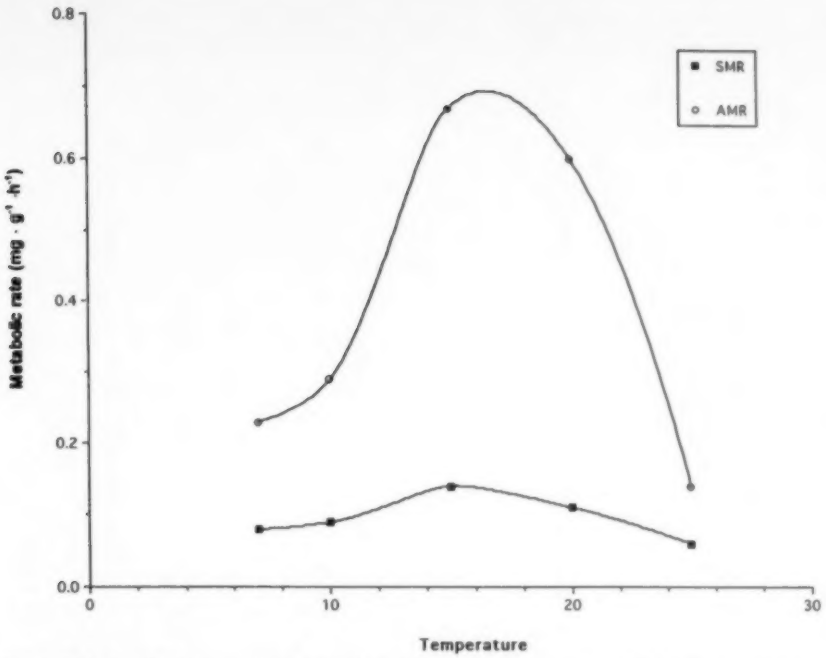

FIG. 2. Relationship between standard and active metabolic rates of a 1.92-g larval sea lamprey acclimated to 7, 10, 15, 20, and 25°C estimated by equation 6 in the text. Standard and active metabolism were estimated by substituting swimming speeds of 0 $cm \cdot s^{-1}$ and maximum prolonged velocities into equation 6.

(Potter and Beamish 1975). Species in this guild, which includes white sucker (*Catostomus commersoni*), northern pike (*Esox lucius*), walleye (*Stizostedion vitreum*), sauger (*Stizostedion canadense*), and yellow perch (*Perca flavescens*), have final preferenda between 20 and 28°C and ultimate upper incipient lethal temperatures between 28 and 34°C (Hokanson 1977). Jobling (1981) reported empirical relationships between growth optima, lethal, and final preferred temperatures of fish, one of which predicts a final preferendum of 22.6°C for larval sea lamprey. This prediction is within the range of variability around our estimate of the final preferendum and thus provides some confidence in the reliability of our data. However, Jobling's (1981) analysis may be confounded by ontogenetic effects, as he used data from different life stages of the same species in some cases. For example, the optimal growth temperature for adult sea lamprey and the final preferred and lethal temperatures of the larvae were included in his data set.

Our statistical analyses of the preferred temperature data were applied descriptively rather than to test specific a priori hypotheses so that we could compare our results with studies on other fish species because there are no comparable lamprey studies. The significant positive slope of the summer regression indicates that preferred temperature is influenced by acclimation temperature. These findings are consistent with the literature on preferred temperatures of temperate freshwater fish, but the slope of the regression (0.192) is lower than the slopes of similar regressions reported for cyprinids (0.56–0.67), centrarchids (0.46–0.68), ictalurids (0.58–0.84), percids (0.41), or salmonids (0.54) by Cherry et al. (1975) and Mathur et al. (1981). During winter tests using horizontal or vertical gradients, acclimation temperature has been reported to have no effect on the preferred temperatures of lake trout (*Salvelinus namaycush*) (McCauley and Tait 1970) and a negative effect on the preferred temperatures of brook trout (*S. fontinalis*) (Sullivan and Fisher 1953) and rainbow trout (*Oncorhynchus mykiss*) (Garside and Tait 1958). Based on the 95% CLs around the slope of the regression (−0.086 and 0.37) in the winter data for sea lamprey, there is no basis for preferring any of the following hypotheses over another: coefficient of AT > 0, coefficient of AT = 0, or coefficient of AT < 0. However, only 9% of the variation in the lamprey data was explained by acclimation temperature. Larval sea lamprey are less active than other species; thus, temperature selection may not be as precise in a linear gradient.

The final preferendum of 16.8°C in winter (this study) is about 3°C higher than the 13.6°C obtained by Reynolds and Casterlin (1978). The difference in reported values for sea lamprey larvae may be related to experimental methodology, to age, and possibly to behavioral factors. Reynolds and Casterlin used an electronic shuttlebox apparatus in which individual fish have more precise control over the heating and cooling of their body temperature. The larvae tested by Reynolds and Casterlin were obtained from a slower growing northern Lake Huron population and were probably older than those that we tested (3+ yr). Reynolds and Casterlin also tested their larvae individually whereas we used groups so the possibility that behavioral interactions influenced preferred temperature cannot be ruled out.

There are few estimates of standard or active metabolic rates in larval lamprey. Most of the available data are measurements of routine metabolism (Table 4) which show that lamprey oxygen consumption is low compared with other

aquatic vertebrates of comparable mass (Hill and Potter 1970). Hill and Potter (1970) and Bourque (1974) reported oxygen consumption for larvae of two lamprey species burrowed into artificial substrates and argued that these data approximated standard metabolism because activity, although not explicitly quantified in either study, was limited while a larva was burrowed. There are no significant differences ($p > 0.05$) among the data reported by Hill and Potter (1970), Bourque (1974), and the standard rates reported in this study for larval sea lamprey (Table 4) after taking into account size differences (Holmes 1992). Leach's (1946) data for *P. marinus* and *Ichthyomyzon fossor* differ significantly ($p < 0.05$), reflecting some unspecified level of activity.

The compound Arrhenius model was applied to the sea lamprey metabolism data in an empirical and descriptive manner. The model provides a reasonably good fit to these data, judging by the R^2 value (0.66) and considering the highly variable nature of the data set. The shape of the active metabolism curve in Fig. 2 is consistent with expectations based on findings for sockeye salmon (*Oncorhynchus nerka*) (Brett 1964, 1971), largemouth bass (*Micropterus salmoides*) (Beamish 1970), and rainbow trout (Dickson and Kramer 1971), but the standard metabolism relationship is not consistent with empirical evidence from these studies. A similar relationship was obtained using a more conventional regression–graphical analysis of these data (Holmes 1992). We offer two hypotheses to explain this phenomenon: first, the unimodal shape of the SMR curve may simply reflect stress imposed by our protocol; or second, lampreys have a smaller gill area per unit mass than most teleost fish (Randall 1972), so the depression of SMR at the higher temperatures may be an indication of inefficient gas transfer and some anaerobic metabolism.

Our coefficient estimates for the metabolism model (Table 3) are probably influenced by the narrow breadth of the sea lamprey data set. For example, the mass coefficient (−0.66) in our model falls between reported values for standard (−0.24; Robinson et al. 1983) and active metabolism (−0.80 to −0.98; Beamish 1970) relationships. The size range of sea lamprey (1–3 g) may not have been sufficiently large to accurately determine the mass coefficient, since a range in excess of one logarithm cycle (e.g., 10–100 g) is generally considered necessary to describe the mass – metabolic rate relationship (Brett 1964, 1971; Beamish 1970). In contrast, the swimming speed coefficient (1.51) is higher than coefficients reported for more active species such as largemouth bass (0.55–0.74; Beamish 1970), possibly because the range of swimming speeds (up to 1.5 body lengths (BL)·s^{-1}) is much lower than the range of speeds at which more active fish are capable of maintaining prolonged activity (up to 6–10 BL·s^{-1}). The temperature coefficients (A, B, C, D) have the same magnitude and exhibit the same general pattern among themselves for standard metabolism (*A* and *B*) and active metabolism (*A*, *B*, *C*, *D*) reported by Regier et al. (1990) and P. Lin and H.A. Regier (unpublished data) for other fish species.

Based on these somewhat weak tests of the relevance of the compound Arrhenius model, we suggest that the model has some explanatory capabilities with respect to the physiological responses of larval sea lamprey to temperature. The tests consisted of the statistical goodness-of-fit, the position of maximum scope for activity relative to temperature, general consistency of the magnitude of the coefficients with estimates for data in which temperature did not vary, and consistency with the general pattern among the model coefficients reported in the literature. Also, compared with the conventional two-step regression–graphical approach, the compound Arrhenius model has logical consistency, using all of the data available in one calculation step to predict metabolic rate at a particular temperature. We submit, however, that our results be used with caution because of the difficulties in ascertaining the reliability of our data and because the thermodynamic implications of the coefficients have not been fully explored with respect to their application to fish phenomena (P. Lin and H.A. Regier, unpublished data).

The relationships that we have found for larval sea lamprey are weak because of variation in the data introduced by the experimental protocol. For example, based on tests to determine lethal temperatures, Potter and Beamish (1975) suggested that larval lamprey are stressed if not allowed to burrow under experimental conditions; this was not possible in our studies. Shocking also stresses fish, producing statistically high metabolic rates (Brett 1964) which we attempted to account for by correcting the data statistically. The weak swimming performance of the larval sea lamprey (Table 2) compared with that of salmonids, which can maintain speeds of 6–10 BL·s^{-1} (Webb 1975), also likely contributed to the high variability in our data, as the equipment was originally designed for use with active fish such as salmonids rather than the sedentary animals used here. Hence, we judge that our data are biased upwards by the hidden effects of stress imposed by the experimental protocol, but we believe that the patterns revealed in our data are an accurate representation of the thermal responses of larval sea lamprey.

The final preferred temperature of larval sea lamprey is estimated to be 20.8°C (Fig. 1) and is close to the temperature at which maximum scope for activity was observed, approximately 19°C (Fig. 2). These findings agree with empirical assessments of temperature relationships in fish (Jobling 1981; McCauley and Casselman 1981) and more specific correlations between behavioral and physiological temperature optima reported for sockeye salmon (Brett 1971), largemouth bass, and bluegill (*Lepomis macrochirus*) (Magnuson et al. 1979). These observations support the generalization that fish select temperatures that afford them the maximum ability to do work (e.g., grow, swim) where those temperatures are available in their environment.

Optimal Thermal Habitat

The fundamental thermal niche was approximated by Magnuson et al. (1979) as a 4°C temperature range centered symmetrically on a species' final preferendum. Christie and Regier (1988) pointed out that the temperatures selected by fish in the field are consistently lower than predicted by the final preferenda because of the influence of other factors and proposed using a 4°C range skewed down about 1° from the final preferendum, i.e., +1° and −3° around the final preferendum, to accommodate these influences. They provided statistical evidence that characterized the thermal environment available to four commercially important fish species in 21 large North American lakes using this skewed optimal temperature range and developed time-integrated measures of thermal habitat area and thermal habitat volume. Following Christie and Regier (1988), we propose the funda-

mental thermal niche of larval sea lamprey to be the range of temperatures between 17.8 and 21.8°C.

Optimal thermal habitat can be calculated from published seasonal temperature and discharge data for a given stream. For example, the sum of daily mean flows during a normal summer when temperatures fall within the range of the larval lamprey's thermal niche would yield a measure of thermal habitat volume. The benthic orientation of larval sea lamprey suggests that the bottom area within the optimal temperature range (i.e., thermal habitat area) is a more appropriate measure of thermal habitat than the volume of water flowing over a locale, and it may also account for the effect of differences in stream flow on thermal habitat. Habitat area in a stream is not normally measured systematically during stream surveys of established lamprey populations in the Great Lakes. However, the findings of Young et al. (1990) concerning substrate preferences might be used to estimate, approximately, the area of suitable habitat in streams until more complete habitat studies are undertaken.

The applicability of these measures of optimal thermal habitat could be tested by using them as predictor variables in comparative studies against dependent variables such as life-history parameters. Patterns in growth rates with mean annual air temperature (age at 120 mm; Holmes 1990) and mean annual water temperature (mean length at age 2+; Young et al. 1990) have been established for larval sea lamprey distributed geographically from the cold northern limit of their distribution around Lake Superior to the warm southern limit (within the Great Lakes basin) around Lake Erie. These patterns could be explored more fully using the measures of environmental temperature proposed here. If this characterization of temperature proves to have relevance to life-history parameters, then it can be used to comparatively assess the productive capacity of established populations in different streams.

If the relationships and interpretations reported here survive further testing, then they may have several applications with respect to the integrated management of sea lamprey in the Great Lakes. First, such measures may be used to map habitat within a stream. Quantifying the effects of habitat variables such as substrate (Young et al. 1990) and temperature on habitat selection and abundance will improve survey methodology by providing more definitive sampling protocols. Second, these relationships could be used to assess whether manipulating stream temperature would reduce larval production (S.H. Smith, cited in Lamsa et al. 1980) sufficiently to be considered as an alternative control strategy. Finally, the relationships suggested here could be used to provide a more definite assessment of the effects of climatic change on sea lamprey populations than that initially provided by Holmes (1990).

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Growth Models with Corrections for the Retardative Effects of Tagging

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Length increment data from mark–recapture experiments are commonly used to obtain information on animal growth, assuming that tagging does not affect the growth of marked animals. The assumption is violated in many studies, but the effects of tagging on growth and estimates of growth parameters have not been and cannot be examined without appropriate models. This paper describes a model allowing quantification and estimation of the retarding effects of tagging on animal growth simultaneously with growth parameters in all existing growth models, reduction or elimination of biases in growth parameters induced by tagging, and relaxation of a key assumption in growth analysis using length increment data. A special case of this model was applied to simulated data and to tagging data from a centropomid perch (*Lates calcarifer*) to demonstrate its general utility. Tagging was inferred to have stopped the fish growth for 36.44 d (ASE = 12.70 d) if von Bertalanffy growth is assumed, but the period of recovery from tagging seemed size or age independent within the size range studied. If tagging retards animal growth, L_∞ is slightly overestimated and K underestimated for unbiased data. Potential applications and limitations of the model are also discussed.

Les données sur l'augmentation de la longueur provenant d'expériences de marquage et de recapture sont couramment utilisées pour obtenir des informations sur la croissance des animaux, en supposant que le marquage n'affecte pas la croissance des animaux marqués. Dans de nombreuses études, l'hypothèse bien souvent n'est pas respectée, mais les effets du marquage sur la croissance et les estimations des paramètres de croissance n'ont pas été et ne peuvent être étudiés sans modèles appropriés. Le présent article décrit un modèle qui permet de mesurer et d'estimer les effets de ralentissement du marquage sur la croissance de l'animal en même temps que les paramètres de croissance dans tous les modèles de croissance existants, la réduction ou l'élimination des biais dans les paramètres de croissance induits par le marquage, et la formulation d'une hypothèse clé dans l'analyse de la croissance au moyen de données sur l'augmentation de la longueur. Un cas spécial de ce modèle a été appliqué à des données simulées et à des données sur le marquage obtenues chez un centropomide (*Lates calcarifer*) pour démontrer son utilité générale. On a conclu que le marquage avait mis un frein à la croissance du poisson pendant 36,44 jours (ASE = 12,70 jours) si l'on suppose une croissance selon l'équation de von Bertalanffy, mais la période de rétablissement au marquage a semblé être dépendante de la taille ou de l'âge dans la plage de tailles étudiées. Si le marquage ralentit la croissance de l'animal, la valeur L_∞ est légèrement surestimée et la valeur K sous-estimée pour des données non biaisées. On traite également des applications et des limites potentielles du modèle.

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Knowledge of the growth of animals has been used directly or indirectly to estimate their age composition, natural mortality, survivorship, and intrinsic rate of increase and to make inter- and intraspecific comparisons (e.g., Roff 1984). It may be obtained by analysis of length at age, modal temporal progression of length composition, and/or length increments from a mark–recapture experiment (e.g., Sainsbury 1980; Schnute and Fournier 1980; Schnute 1981; Kirkwood 1983; Kirkwood and Somers 1984; Smith and McFarlane 1990; Hampton 1991; Buckworth 1992). Use of length at age poses problems because it requires exact age data, which are sometimes unavailable. Attempts at estimating age through counting regular protein and calcium depositions in scales, otoliths, vertebrae, and fin rays occasionally result in imprecise and inaccurate age data, with little or no control over ageing errors. Modal progression analysis offers a way of utilizing length data, but growth parameters estimated this way are often unreliable because of the subjectivity in constructing length frequency distributions from the often biased samples of length data (e.g., Silverman 1986), in identifying age classes, and in making assumptions about distributions of length at age. Use of analysis of length increments is widespread, particularly with the miniaturization and increased utility of tags (e.g., Hancock 1989; Parker et al. 1990), which are defined here as markers placed by man on or in animals.

In growth studies, length increment analysis has four basic assumptions (e.g., Kearney 1989): (1) behaviour of the animals tagged is unaffected by tagging; (2) tagging neither impedes nor facilitates their growth; (3) recoveries or recaptures are independent of animal sizes; and (4) size-specific recruitment and all measurement errors are accounted

for. There is, of course, also the inconsistent use of dependent and independent variables in analysing length at age and length increment data (Francis 1988). Various aspects of tagging effects have been examined (e.g., Davis and Reid 1982; Hill and Wassenberg 1985; Fitz and Wiegert 1991; Montgomery and Gray 1991; Primavera and Caballero 1992), but those studies were based generally on short-term and/or laboratory experiments, giving little useful information on how tagging affects growth under natural conditions. Indeed, one of the most serious and yet untested assumptions concerns the effects of tagging on the growth of animals. Although some tags may distort the growth of tagged animals for any period of time following tagging, animals recaptured shortly after tagging have been observed to grow negatively or retardedly (e.g., Manire and Gruber 1991; Scheirer and Coble 1991; McAllister et al. 1992). Unfortunately, such observations are usually ignored or not commented on in growth analysis and ensuing applications mainly because of difficulties in quantification and interpretation. The question, then, is not "Does tagging affect the behaviour and growth of animals?", but "Is the level of such distortion acceptable?", and "Can its effects be quantified or compensated for?" (Thresher 1989). Unless those questions are answered, convincingly, and unless the long- and short-term effects of tagging on behaviour and growth are assessed, reliability of conclusions from any study involving tags is in doubt. Some workers (e.g., Kirkwood 1983; Hampton 1991; Buckworth 1992) recognized and tried to overcome tagging effects on growth by rejecting early-captured animals in the belief that such rejection would eliminate or at least reduce the biases induced. Unfortunately, this approach does not work and can only lead to a loss of information because the effects of tagging, if any, become a part of subsequent growth history. The only practical way to tackle the problem is to quantify tagging effects together with growth parameters. In this paper, I will develop a method applicable to all existing growth models (e.g., von Bertalanffy, Gompertz, and logistic) by which some measure of tagging effects is defined and can be estimated simultaneously with growth parameters, thus eliminating or at least reducing the biases they cause, and relaxing a key assumption in analysing length increment data from tagging studies. The models developed here are very general to stimulate further development and refinement and to allow flexibility. I will, however, present a useful special case of these models and apply it to both simulated and real data.

Model Formulation

Tagging effects on growth of animals are seen here as a resultant of many processes (e.g., capture, handling, tagging, and release) and probably remain even after possible resumption of normal growth. I will refer to all of these processes as tagging unless otherwise specified. Tagging affects animal growth positively, antagonistically, or neutrally regardless of the mechanisms involved. Data on the positive effects of tagging must be available for animals treated or "tagged" with growth-promoting hormone(s) as in aquaculture. Antagonistic effects are either retardative or negative; the former is applicable generally to such unshrinkable quantities as length of fish (e.g., Manire and Gruber 1991; Scheirer and Coble 1991; McAllister et al. 1992), while the latter most likely to shrinkable quantities, such as weight or mass of animals (e.g., McAllister et al. 1992). Neutral effects of tagging can be viewed as a special case of the other two cases. This paper deals with length-based growth models in the case of the well-documented retardative effects of tagging (e.g., Manire and Gruber 1991; Scheirer and Coble 1991; McAllister et al. 1992). In the following, the word retardative is omitted for convenience of description, and recovery period and tagging effects may be used interchangeably in contexts where their respective meanings are clear.

Now, let a general model of animal growth be given by the continuous function

(1) $L_t = g(t)$

for length L_t at age t and

(2) $\Delta L = \Delta g(L_t, \Delta t)$

for length increment ΔL during a period of time at liberty Δt for initial length L_t. In a tagging experiment, an individual is assumed to grow following equation 1 or 2 (and would do so if untagged) until it is captured, marked/tagged, and released at $t = t_1$. After, and as a result of, tagging, the growth of this individual stops for a period of time, τ, until $t = t_1 + \tau$, when normal growth is resumed. The growth of such an animal is described by

$$(3)\quad L_t = \begin{cases} g(t - t_0) & t_0 \le t \le t_1 \\ g(t_1 - t_0) & t_1 \le t \le t_1 + \tau \\ g(t - t_0 - \tau) & t_1 + \tau \le t \end{cases}$$

or in difference form by

$$(4)\quad \Delta L = \begin{cases} 0 & 0 \le \Delta t \le \tau \\ \Delta g(L_t, \Delta t - \tau) & \Delta t \ge \tau \end{cases}$$

where τ is also referred to as the period of recovery and is a measure of the extent of tagging effects. These are the general growth models corrected for tagging effects.

Various growth models (e.g., von Bertalanffy, Gompertz, and logistic) have been proposed for fitting growth data. It should be noted in choosing $g(t)$ that deviation of growth from it may be natural and, if large enough, may destabilize the general approach presented above. It is then essential that $g(t)$ be chosen cautiously by trying a variety of general and flexible growth models such as Sainsbury's (1980) and Schnute's (1981) to reduce such destabilizations.

τ must depend on size and species of animals and on tag types, but it may be too small to be statistically detected in some species or size classes of a species for certain tags, while relatively large in others, especially for physically very damaging tags. Functional forms of τ should therefore be explored in terms of L_t and other variables and parameters, especially in the presence of supportive data. For example, τ may be a constant, being independent of size and age of the tagged animal. It may also be a function of size and age, i.e., $\tau = \tau(L_t)$. For example, τ may decrease in animals of vulnerable early age classes, or increase in older age classes because of their slower recovery, or even have an extreme value because juveniles may be either more robust or vulnerable to tagging than either early or adult stages. Other hypotheses can, of course, be formulated and tested.

There are at least two ways of incorporating τ into $g(t)$ and $\Delta g\ (L_t, \Delta t - \tau)$. One could work from real-time models (e.g., equation 3) to models in difference equations (e.g.,

equation 4), or vice versa, being constantly guided by biological principles. The former route is, however, often easier but probably less appealing intuitively than the latter, which involves solving difference equations. One cannot simply write down a difference equation which does not make sense in real time and proceed with the estimation. Finally, continuity conditions must be observed in the segmented real-time models to reduce the number of parameters and to avoid model overparameterization.

A useful special case of these general growth models corrected for tagging effects is the commonly used von Bertalanffy growth equations (VBGEs). These have nonetheless been strongly criticized by various workers (e.g., Knight 1968; Roff 1980) and are given by

$$(5)\quad L_t = L_\infty(1 - e^{-K(t-t_0)})$$

$$(6)\quad \Delta L = (L_\infty - L_t)(1 - e^{-K\Delta t}) \text{ (Fabens 1965)}$$

$$(7)\quad L_t = \begin{cases} L_\infty\,(1-e^{-K(t-t_0)}) & t_0 \le t \le t_1 \\ L_\infty\,(1-e^{-K(t_1-t_0)}) & t_1 \le t \le t_1+\tau \\ L_\infty\,(1-e^{-K(t-t_0-\tau)}) & t_1+\tau \le t \end{cases}$$

$$(8)\quad \Delta L = \begin{cases} 0 & 0 \le \Delta t \le \tau \\ (L_\infty - L_t)(1-e^{-K(\Delta t-\tau)}) & \Delta t \ge \tau \end{cases}$$

with von Bertalanffy parameters (K, L_∞, t_0).

Data and Analysis

Two data sets were used to demonstrate the general utility of the special case model. The first data set of 300 points was simulated using equation 8 for L_∞ = 40 cm, K = 1.65·yr^{-1}, τ = 0.2 yr, $L_t \sim U(0,L_\infty)$ cm, and $\Delta t \sim U(0,2)$ yr with the model error $\epsilon \sim N(0,0.1^2)$ cm and reanalysed to demonstrate the general utility of the growth models corrected for tagging effects. The second data set analysed using equation 8 was that of Davis and Kirkwood (1984) on barramundi (*Lates calcarifer*), a protandrous fish commonly found in estuaries and other coastal areas of the Indo-West Pacific (Griffin 1987). Over 4933 barramundi with a body total length range of about 10–100 cm were captured by a combination of lure fishing, tidal trap, seine, and gill net, measured to the nearest centimetre, tagged with the then commonly used, but apparently physically and physiologically damaging, Floy FT-2 Dart tags for fish >35 cm and FD-67 Anchor tags for fish <35 cm, and released in rivers flowing into the Van Diemen Gulf and the Gulf of Carpentaria of northern Australia between August 1977 and June 1980 (Davis and Reid 1982). Of those tagged, 312 fish of a total length of 23–92 cm (mean = 60 cm, SD = 13 cm) were recaptured, but only 309 were used in the analysis below due to incomplete recapture information. The time at liberty ranged from 0 to 932 d with a mean of 219 (SD = 211) d, and the length increment from 0 to 35 cm with a mean of 6 (SD = 8) cm. The data set was admittedly not the best choice because of the limited size range of recaptures for a fish which may reach 150 cm in total length because of the possible bias introduced by using different tags on juveniles and adults and because of the possible additional variability arising from the different capture techniques. However, the data are still useful to illustrate the method. They were first fitted using the nonlinear least squares method into equation 8 with $\tau = \tau' + bL_t$, where τ' and b are parameters to be estimated, to explore possible effects of L_t on the recovery period. The model was then tested using likelihood ratio test (Draper and Smith 1981) against a reduced model with H_0: $b = 0$, which in turn was tested against a still more reduced model with H_0: $\tau = 0$.

Results

Analysis, using equation 8, of the data set of 300 points simulated using the model itself for L_∞ = 40 cm, K = 1.65·yr^{-1}, τ = 0.2 yr, $L_t \sim U(0,L_\infty)$ cm, and $\Delta t \sim U(0,2)$ yr with the model error $\epsilon \sim N(0,0.1^2)$ resulted in L_∞ = 40.02 (asymptotic standard error ASE = 0.01) cm, K = 1.641 (ASE = 0.003)·yr^{-1}, and τ = 0.1983 (ASE = 0.0005) yr (R^2 = 0.99997, $F_{3,297}$ = 2967432.68, $P \le 0.0000$, n = 300). There is a remarkable similarity between estimates of various parameters and those that were used to generate the data, suggesting that the growth models corrected for tagging effects work well if the data are unbiased. In contrast, analysis of the same data using the classic VBGE of Fabens form gave L_∞ = 41.42 (ASE = 0.28) cm and K = 1.085 (ASE = 0.027)·yr^{-1} (R^2 = 0.98856, $F_{2,298}$ = 12878.75, $P \le 0.0000$, n = 300), showing slight discrepancies between estimate of L_∞ and the parameter itself and a very large difference between estimate of K and the parameter value itself (K = 1.65·yr^{-1}).

Lates calcarifer tagging data were first fitted into equation 8 with $\tau = \tau' + bL_t$, where τ' and b are model parameters, and resulted in L_∞ = 110.76 (ASE = 9.29) cm, K = 0.277 (ASE = 0.063)·yr^{-1}, τ' = 0.0900 (ASE = 0.1006) yr, and b = 0.000038 (ASE = 0.001587)yr·cm^{-1} (R^2 = 0.793, $F_{4,305}$ = 299.99, $P \le 0.0000$, n = 309), with a residual sum of squares of 6861.54 cm^2 and a mean residual sum of squares of 22.50 cm^2. The size dependency parameter b was not significantly different from zero judging from an estimate of its asymptotic standard error, indicating that the size at capture was not a determinant of the recovery period in the size range investigated.

Fitting the same data into a reduced model with H_0: $b = 0$ gave L_∞ = 110.48 (ASE = 8.52) cm, K = 0.281 (ASE = 0.059)·yr^{-1}, and τ = 0.0998 (ASE = 0.0347) yr (R^2 = 0.797, $F_{3,306}$ = 401.31, $P \le 0.0000$, n = 309), with a residual sum of squares of 6861.50 cm^2 and a mean residual sum of squares of 22.42 cm^2. Thus the estimate of τ was significantly different from zero, indicating that the effects of tagging retarded the growth of the fish for about 36.44 d. A likelihood ratio test of the full model against this reduced model cannot be done because the reduced model actually explained a greater proportion of the variability in the data (see the two residual sums of squares above), indicating overparameterization of the full model.

For the classic VBGE of Fabens form, the regression analysis resulted in L_∞ = 113.75 (ASE = 9.71) cm and K = 0.236 (ASE = 0.049)·yr^{-1} (R^2 = 0.792, $F_{2,307}$ = 585.12 $P \le 0.0000$, n = 309), with a residual sum of squares of 7036.23 cm^2 and a mean residual sum of squares of 22.92 cm^2. The above reduced model was tested against this more reduced model with H_0: $\tau = 0$, giving $F_{1,306}$ = 7.79 and $P < 0.0055776$, suggesting that the classic VBGE of Fabens form is statistically significantly different from that with a constant τ.

Discussion

Recognition, definition, and introduction of tagging effects into growth models are essential for reducing or eliminating biases induced by tagging in the estimates of growth parameters. This work presents a general method by which retardative effects of tagging are defined, introduced into growth models, and estimated, thus forming a basis upon which quantitative information on the growth of animals subject to tagging can be obtained. This relaxes a key assumption in analysing length increment data from tagging studies. Analysis of the simulated data set has shown the general utility of the models. The significance of this work also lies in the various specific models (equations 5–8) developed and used to fit the data, while other specific models may be derived easily following this method. It should be mentioned that the method allows only short-term effects of tagging to be evaluated; evaluation of long-term effects would probably require prior knowledge of growth, which is usually unavailable and the object of most growth studies.

The models formulated above have many additional applications. An obvious one would be the examination of the effects of artificial conditions on the growth of animals, such as those first living naturally and then reared in the laboratory. Secondly, growth of cultured species treated with growth-inhibiting hormones can be modelled by comparing growth patterns prior to and after hormonal treatment. The models may also be used to describe single growth cessations, induced for instance by reproduction, in natural animals and would be more useful in this context for animals of shorter longevity than those of longer ones because in the latter, cessations of growth if present may be smoothed, and for females than males because males are often less affected by reproductive events and would follow a smoother growth curve than females. Since natural cessations of growth in animals are compounded by tagging effects, the models would be more useful when the variability from various sources is controlled. Finally, the recovery period and effects of tagging may be examined by formulating hypotheses in relation to size of tagged animals and various other parameters. For example, that the estimate of the parameter τ as a function of size and age, i.e., $\tau = \tau' + bL_i$, was insignificantly different from zero in *L. calcarifer* as above may suggest that the effects of tagging are not size or age specific within the size range of the animals tagged.

The effects of tagging would lead to a gross underestimation of K and a slight overestimation of L_∞ for unbiased data if the von Bertalanffy equation is used for growth descriptions. This is clearly demonstrated in analysing the simulated data set, where L_∞ is overestimated by a marginal 3.50% and K underestimated by as much as 33.90%. The results obtained for *L. calcarifer* showed that L_∞ would be overestimated by 2.97% and K underestimated by 16.10% if the growth models, corrected for tagging effects, were the correct ones while the Fabens form were used for growth description. It should be remembered, however, that the results with this fish are illustrative and should be treated with caution because of biased data, where only relatively small-sized (<1 m) animals were tagged, recaptured, and used in the analysis, while the species may reach a body length of about 150 cm. In this case, alternative interpretations of L_∞ by Schnute and Fournier (1980) and Francis (1988) as functions of reference lengths at age or length increments for given initial length and time at liberty may well apply.

There are limitations to the above models. In most growth analyses, data are collected not from a single individual, but from a group of animals, as with the *L. calcarifer* tagging data. In this case, animals of a cohort or population have been assumed but cannot be expected to have an identical set of growth (e.g., Kirkwood and Somers 1984; Smith and McFarlane 1990; Hampton 1991) and recovery (from tagging) parameters due to individual variability in growth and recovery after tagging. Indeed, both types of parameters should have been incorporated in the model. However, there would have been too many parameters and assumptions about their distributions for most data sets, while assumptions regarding distributions of those parameters could be removed by treating the problem as an inverse one (e.g., Burr 1988). This inverse approach would again involve estimation of large numbers of parameters and is usually computationally expensive. Nonetheless, the results obtained here and elsewhere based on the parameter constancy assumption should be interpreted cautiously. A similar problem is that tagging may permanently affect growth parameters such as K and/or L_∞, but this problem cannot be solved currently due to a lack of data on how tagging affects growth of animals. Finally, the nonlinear least squares approach to model fitting adopted in this paper assumes that the variance of residuals is independent of L_i and Δt. If this is not the case, there may be problems with the results of significance tests (Schnute 1991) and the error structure should be modelled accordingly (e.g., Sainsbury 1980; Schnute 1991).

Although the effects of tagging have been defined and estimated in terms of recovery period and length of animals, alternative definitions, measures, and formulations are available. While length and many other linear dimensions are unlikely to "shrink", weight or mass loss can be considerable in many animals. For instance, the crested newt *Triturus cristatus* (Glandt 1981) and the air-breathing fish *Heteropneustes fossilis* (Marian et al. 1982) can lose up to 4% of body weight under adverse food conditions, and hence possibly as a result of tagging. In this sense, weight may be a better and more sensitive descriptor of tagging effects than linear measures. Since both negative (e.g., Hosie and Ritz 1989) and positive growth from tagging (e.g., by use of growth promoting hormones) may occur, formulations of growth models corrected for positive, antagonistic, and neutral effects of tagging should be developed with the above models as special cases.

Finally, in mathematical modelling, there are always dangers of overparameterization by attempting to extract too much information from the data and of underparameterization which may lead to a loss of interpretive meanings of models. The models developed above are comparable in the number of parameters with some other forms (e.g., seasonal growth components) of growth models and can be directly used to examine the effects of tagging in many existing data sets without any further data requirements. Also, they do not impose more constraints on sampling design and quality of data, although, as for any other models, more and better data would give more useful and meaningful results.

In summary, this work presents a general method by which retardative effects of tagging are defined, introduced into

growth models, and estimated. It forms a basis upon which quantitative information on the growth of animals can be obtained subject to tagging effects. Finally, it relaxes a key assumption in analysing length increment data from tagging studies. Some specific models were developed and tested with both real and simulated data which revealed the implications of ignoring the biasing effects of tagging on estimates of growth parameters. The model has limitations, but also a number of possible applications.

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Foraging Movements in Relation to Morphology, Water-Column Use, and Diet for Recently Emerged Brook Trout (*Salvelinus fontinalis*) in Still-Water Pools

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McLaughlin, R.L., J.W.A. Grant, and D.L. Kramer. 1994. Foraging movements in relation to morphology, water-column use, and diet for recently emerged brook trout (*Salvelinus fontinalis*) in still-water pools. Can. J. Fish. Aquat. Sci. 51: 268–279.

Recently emerged brook trout (*Salvelinus fontinalis*) foraging is still-water pools varied considerably in the proportion of time they spent moving, speed while moving, and distance travelled during pursuit of prey. Trout that exhibited a high proportion of time spent moving, fast speeds while moving, and long pursuit distances directed more foraging attempts toward the upper portion of the water column, ate more insect prey and fewer crustaceans, and made more foraging attempts per minute than trout that exhibited a low proportion of time spent moving, slow speeds while moving, and short pursuit distances. The variation in movement was related less strongly to spatial heterogeneity in the locations (pool sizes, water depths) where individuals were observed. It was not significantly related to measures of temporal variation in the environment (date, time-of-day, water temperature), body size and shape, or hunger (the amount of food in the stomach). In these clear, shallow pools, differences in the spatial distribution and behaviour of prey types apparently facilitate divergent foraging behaviour and diet among the trout. Our findings are valuable for understanding the development and maintenance of the individual specializations in water-column use and diet reported previously for several salmonids.

On a observé des différences considérables chez des ombles de fontaine (*Salvelinus fontinalis*) récemment émergés, cherchant leur nourriture dans les fosses d'eau tranquille, en ce qui concerne la proportion de temps passé en déplacement, la vitesse de déplacement et la distance parcourue à la poursuite des proies. Les ombles qui passaient une proportion élevée du temps à se déplacer, qui se déplaçaient rapidement et parcouraient de longues distances en poursuite orientaient un plus grand nombre de tentatives de recherche de nourriture vers la partie supérieure de la colonne d'eau, mangeaient plus d'insectes et moins de crustacés, et faisaient plus de tentatives par minute que les ombles chez qui on observait des résultats plus faibles pour ces facteurs. La variation dans le déplacement était moins fortement liée à l'hétérogénéité spatiale des endroits (taille des fosses, profondeur de l'eau) où les individus étaient observés. Elle n'était pas en rapport significatif avec les mesures de la variation temporelle de l'environnement (date, heure du jour, température de l'eau), avec la taille et la forme du corps ni avec la faim (quantité de nourriture dans l'estomac). Dans ces fosses claires et peu profondes, les différences dans la répartition spatiale et le comportement des types de proie semblent faciliter l'existence de différences dans le comportement de recherche de nourriture et dans l'alimentation parmi les ombles. Nos résultats sont utiles pour comprendre le développement et le maintien de la spécialisation individuelle dans l'utilisation de la colonne d'eau et dans l'alimentation qui a été déjà signalée pour plusieurs salmonidés.

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Behavioural variation observed among individuals in the field may reflect a variety of phenomena ranging from short-term roles adopted by a flexible phenotype to stable, repeatable differences possibly related to different genotypes or fixed alternative phenotypes (Partridge and Green 1985; Clark and Ehlinger 1987). In a proximate sense, the behavioural variation among and within individuals is expected to represent a complex interaction between an animal's phenotypic attributes and the environmental variability it encounters (Ringler 1983). Phenotypic attributes include behavioural (e.g., experience, learning ability), morphological (e.g., body size and shape), physiological (e.g., anaerobic and aerobic capabilities, hunger), developmental, and sex-related characteristics of individuals. Environmental variation includes spatial and temporal differences in temperature, predation risk, the availability of food, mates, or space, as well as variation in the actions of other conspecifics. Disentangling the importance of these factors, particularly under field conditions, presents a major challenge. Comparing individuals exhibiting different behaviour can be a valuable step toward identifying phenotypic and

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environmental factors promoting the behavioural variation as well as evaluating whether the variation is important to an individual's growth, survival, and reproduction (e.g., Holbrook and Schmitt 1992).

Short-term observations of recently emerged brook trout (*Salvelinus fontinalis*) foraging in still-water pools along the sides of streams revealed considerable variation in foraging movements (Grant and Noakes 1988; McLaughlin et al. 1992). Some trout spent little time moving, used slow speeds when they did move, and had short pursuit distances during prey capture. Others moved much of the time at relatively high speeds and had longer pursuit distances. These sedentary and active fish were often observed in the same pool at the same time (McLaughlin et al. 1992).

This study evaluates the degree to which this individual variation in foraging movements is related to variation in local environmental conditions and to variation in individual phenotype. The relative importance of environmental and phenotypic factors in shaping patterns of foraging movements in natural environments remains unclear. Earlier interspecific comparisons related a variety of environmental factors to variation in foraging movements, including prey density (Huey and Pianka 1981; Holmes and Recher 1986), predation risk (Johnson and Crowley 1980; Huey and Pianka 1981), habitat structure (Webb 1984; Streams 1987), and diet and microhabitat use (Gatz 1979; Huey and Pianka 1981; Robinson and Holmes 1982; Cooper et al. 1985; Greene 1986). Such studies have also identified physiological and morphological attributes that can influence locomotor ability, and hence foraging movements, including anaerobic and aerobic capabilities (Pough and Taigen 1990; Goolish 1991), relative amounts of different muscle types (Gatz 1979; McLaughlin and Kramer 1991), and body size and shape (Webb 1984). However, these interspecific comparisons focused on historical variation and were not well suited for identifying the factors currently operating in the field. Experimental studies, on the other hand, have been successful in relating many of the same environmental and phenotypic factors to intraspecific variation in patterns of foraging movements (Formanowicz 1982; Sjöström 1985; Savino and Stein 1989; Cloarec 1990; Ehlinger 1990; Johansson 1991; Kieffer and Colgan 1991). However, they have been limited mainly to the laboratory and usually considered only one or two factors at a time.

We used a comprehensive comparison of recently emerged brook trout exhibiting different patterns of foraging movements in the field to determine how strongly the proportion of search time spent moving, the speed while moving during search, and pursuit distance were related to (i) time-of-day and sampling date, (ii) water temperature, (iii) pool surface area and water depth, (iv) body size and shape, (v) the vertical location in the water column where prey were captured and the types of prey eaten, and (vi) the foraging attempts made per unit time and the amount of prey eaten. Time-of-day and sampling date were considered to detect any consistent changes in movement within and among days, possibly in response to aspects of the environment that were not measured directly during our study such as overall food abundance or predation risk. Water temperature was considered because it can limit a fish's swimming capacity (Graham 1949; Beamish 1978). It can also influence the speed maximizing an individual's net rate of energy gain (Ware 1978) because daily food consumption (Elliott 1975) and the energetic cost of swimming at a particular speed (Beamish 1980) can both vary with water temperature. Pool surface area and water depth were considered because pool morphometry might constrain activity levels (Lindsey 1978) or affect the relative abundances of benthic and open-water prey, and thus the foraging behaviour of the fish (Speirs 1974; Lavin and McPhail 1985, 1986). Body size and shape were considered because morphology could either limit an individual's swimming capacity (Beamish 1978; Webb 1984), or affect the cost (energy per unit time) of swimming at a particular speed and hence the level of activity that would maximize an individual's net rate of energy gain (Ware 1978; Dabrowski et al. 1988). The location of capture and the types of prey eaten were considered because earlier studies have linked patterns of foraging movements to diet and microhabitat use (see above). Finally, foraging frequency and the amount of food consumed were considered because hungry animals often feed more actively than well-fed individuals (Beukema 1968; Cloarec 1990). We focused our observations on trout within a narrow range of sizes and ages inhabiting relatively homogeneous, clear, shallow pools to control for large-scale differences in development, age, and spatial heterogeneity. We used partial correlation analysis to control for such variation among and within pools.

Materials and Methods

Study Sites

Data were collected from 1 April to 3 May 1988 and 13 to 25 April 1989 at three sites: one located on the North Branch of the Credit River upstream of Highway 24 near Caledon, Ontario, Canada (43°52′N, 80°00′W), a second 2 km northeast of Erin, Ontario (43°45′N, 80°07′W), on the West Branch of the Credit River, and a third near Limehouse, Ontario (43°38′N, 79°59′W), on Black Creek, a stream flowing into the middle reaches of the Credit River.

Each study site encompassed a 50- to 200-m segment of the stream containing areas of flowing water and 5–14 still, backwater pools along the stream edges. Usually, we alternated between sites on a daily basis, but on three occasions the same site was sampled on successive days. In each case, one of the days involved only a half day of sampling because of poor weather. These fish were too small (see Table 1) and delicate to mark individually, but we believe that the probability of reobserving the same fish was low because we captured 36% of the fish after observation (see below), and our sampling was spaced out among pools and sites over time.

Behavioural Measurements

Observations were made between 09:00 and 17:00 daily unless prevented by poor weather. During data collection, an observer either lay on the streambank, sat on a stool near the edge of a stream, or, infrequently, stood on the bank. Fish usually moved away from the bank or fled to cover when the observer arrived. Before quantifying movement, we waited 5 min for fish to reenter the viewing area and then observed the fish in the area for at least 5 min more and noted whether they were foraging. Following this, we identified all of the fish in view and categorized their activity as "sedentary" or "mobile" based on their behaviour at the time. A foraging fish was then selected as the focal indi-

TABLE 1. Univariate statistics for field measurements of behavioural, environmental, and size variation for recently emerged brook trout.

Variable	$\bar{x}$	SD	Range	Sample Size
Behavioural Measurements				
Proportion of time spent moving	0.43	0.34	0 – 1.0	251
Speed while moving (lengths·5 s^{-1})	2.71	0.99	1 – 4.9	119[ab]
Pursuit distance (fork lengths)	2.94	1.66	0 – 8.5	128[a]
Proportion of feeds at surface	0.46	0.33	0 – 1.0	251
Proportion of feeds in midwater	0.38	0.29	0 – 1.0	251
Proportion of feeds at benthos	0.17	0.24	0 – 1.0	251
Foraging attempts per minute	1.7	1.0	0.1 – 6.1	251
Environmental measurements				
Water temperature (°C)	9.5	2.6	3.0 – 16.0	239
Pool surface area (m^2)	5.1	4.2	0.1 – 10.4	229
Water depth (cm)	8.8	3.6	2.0 – 20.0	246
Body size				
Fork length (cm)	2.4	0.3	1.9 – 3.5	246

[a]Quantified by observer 1 only.
[b]Nine individuals did not move other than to pursue prey.

vidual and its fork length was estimated visually to the nearest 0.25 cm. Waiting 10 min before selecting a focal individual should have minimized any bias toward bold or hungry fish because brook trout this size usually return to their foraging location within 2 min of a disturbance (Grant and Noakes 1987). In addition, the probabilities of selecting a sedentary and mobile fish (0.50 versus 0.50, respectively) were not significantly different from the probabilities of a sedentary and mobile fish being available (0.51 versus 0.49, respectively) ($G = 1.2$, $P > 0.25$, df = 1). After selecting a focal individual, quantification of foraging movements commenced and we tried to collect at least 5 min of data for each focal fish. Actual durations of data collection periods varied depending on the fish's mobility and the sunlight and wind conditions.

Movements of the focal fish were quantified in one of two ways depending on the observer. Observer 1 (R.L.M.) recorded movements at 5-s intervals using a metronome timer and a microcassette recorder. During each interval the observer estimated the distance travelled to the nearest body length. Intervals including acts of flight, aggression, or foraging attempts were noted. Flight entailed rapid movement to cover or a position on the pool bottom and subsequent hiding. Aggression was recorded when the focal fish either chased or was chased by a conspecific. A foraging attempt entailed a sudden change in speed or direction to intercept a potential prey item, whether successful or not. Locations to which the foraging attempts were directed (benthic, midwater, or surface) were also recorded. Intervals not including these behavioural acts were considered to be intervals of search, while intervals including foraging attempts are called pursuits hereafter. Observer 2 (J.W.A.G.) used a continuous time method needed for another facet of our study and recorded on microcassette the time an individual spent moving or stationary and the locations of foraging attempts (benthic, midwater, or surface). Incidences of flight and aggression were also recorded.

Both observation methods provided estimates of the proportion of search time spent moving and the foraging attempts made per unit time. With the first method the proportion of time spent moving was calculated as the proportion of 5-s intervals involving search where the individual moved one fork length or more. With the second method the proportion of time spent moving was calculated as the duration of time spent moving divided by the total duration of the observation period. For 29 fish watched by both observers simultaneously in 1988, the methods provided similar estimates of the proportion of time spent moving and the foraging attempts made per unit time ($r = 0.98$, $P < 0.001$ and $r = 0.96$, $P < 0.001$, respectively). The first method also provided the distance travelled (in fork lengths) per unit time during 5-s intervals when the fish moved one body length or more (the speed while moving) and the distance travelled during pursuits (pursuit distance). The speed while moving was measured and is reported as fork lengths per 5 s and pursuit distance as fork lengths. Because pursuits lasted less than 5 s, the distance moved during intervals including a pursuit could also entail movement related to search. However, our field observations suggested that these distances were reasonable approximations of pursuit distances, and there were noteworthy differences, both in magnitude and variability, between the measurements for moving search and pursuit (see Results: also McLaughlin et al. 1992).

In 1988, we videotaped fish on five occasions and these tapes were used to evaluate the accuracy of our distance estimates. From the tapes, we estimated visually the number of fork lengths moved by an individual fish and then measured the fish's fork length and the distance it traversed and calculated the actual distance moved in fork lengths. One hundred estimates were made. Our visual estimate of distance was a good predictor of the actual distance moved ($r = 0.98$, $P < 0.0001$); the intercept of the regression was not significantly different from 0 ($t = 0.60$, $P > 0.05$) and the regression coefficient was not significantly different from 1 ($t = 0.03$, $P > 0.50$). The median absolute error of our estimates was 8.6%.

Environmental Measurements

Water temperature was recorded immediately following observation of each focal fish, and the observation site was marked. At the end of the sampling day, we measured water

depth and current velocity at each site. Water depth and current velocity represent the average of several measurements made in the area traversed by the focal fish. Current velocity was quantified using a Pigmy Teledyne-Gurley current meter rated for velocities down to 1 $cm \cdot s^{-1}$. We estimated velocities slower than 1 $cm \cdot s^{-1}$ by releasing silt particles at the water surface. Current velocity was recorded as 0 $cm \cdot s^{-1}$ if the particles fell straight down and 0.5 $cm \cdot s^{-1}$ otherwise. Finally, we noted the pool where observations were made and pool sizes (surface areas) were measured several times over the study.

Morphological and Dietary Measurements

Each observer attempted to capture the focal fish at the end of the observation period using two aquarium dipnets. Ninety-one fish observed in still water were captured, measured (fork length ± 0.1 mm), preserved in 10% formalin, and later transferred to 37.5% isopropyl alcohol.

Twenty-three morphological measurements were made on the preserved fish using a dissecting microscope equipped with a micrometer eyepiece (see McLaughlin and Grant 1994). Fifteen fish were remeasured to ensure that the measurements were repeatable (McLaughlin and Grant 1994).

Prey items in the esophagus and stomach of each fish were identified to order, counted, and measured. Intestinal contents of 84 of these fish were also examined for indicators of prey type such as body parts and copepod eggs.

Statistical Analyses

Movement data presented here include fish from only 0 $cm \cdot s^{-1}$ current habitats. All fish were located in the water column and off the stream bottom. The durations of data collection periods ranged from 4.2 to 33.8 min in 1988 ($\bar{x}$ = 10.6 min, N = 184 individuals) and 6.8 to 19.1 min in 1989 ($\bar{x}$ = 14.4, N = 67 individuals). Intervals of flight and aggression accounted for 1% of the total observation time and were removed before calculating the movement variables. Individual means for the speed while moving are based on 1–125 measurements (median = 33.5) and for pursuit distances on 1–65 measurements (median = 10.5). The magnitude of the short-term variation observed for these variables both among and within individuals is presented in McLaughlin et al. (1992).

Several variables were transformed to normalize their distributions as much as possible. The proportion of time spent moving and the proportions of midwater and surface foraging attempts were arcsine-square-root transformed. The number of foraging attempts made per unit time and the number of prey items in the esophagus and stomach were square-root transformed. Water depth, prey length, and all of the morphological variables were $\log_{10}$ transformed.

We do not have all of the information needed to include every individual in every analysis, so sample sizes differ among analyses. Correlations between the movement variables and the environmental and morphological variables were examined before and after adjusting for site and year effects. Data were adjusted for these effects by standardizing all of the variables to mean 0 within each site and year cohort before analysis (i.e., group-centered; see Strauss 1990). Differences between the unadjusted and group-centered analyses were minimal. Analyses using the unadjusted data are presented in the sections relating to environmental variation and to diet composition. Group-centered analyses are presented in the section relating morphological variation to movement because preliminary analyses revealed small, significant morphological differences among the site and year cohorts.

Descriptive statistics for the behavioural, environmental, and size measurements used in our analyses are summarized in Table 1. Fork lengths measured in the field were used for fish that were captured, and visual estimates of fork length were used for fish that were not captured. Visual estimates of fork length (EFL) provided reasonably good predictions of the measured fork lengths (MFL) (regression equation: MFL = 0.09 + 0.95·EFL; N = 85, r^2 = 0.67, $P < 0.0001$).

Predictions regarding morphology were derived from several papers relating morphology to locomotion in fishes. Larger trout were expected to be more active than smaller trout (Ware 1978; Fuiman and Webb 1988). After statistically controlling for standard length, individuals that spent more time moving were expected to have deeper anterior body depths to decrease resistance, and deeper caudal fins to increase thrust, than individuals that spent less time moving (Webb 1984, 1988; Webb and Weihs 1986). After controlling for the depth of the caudal fin, fish that spent more time moving were also expected to have narrower depths at the caudal peduncle to reduce energy losses during recoil than less active fish.

We tested for individual variation in diet composition using a $\varkappa^2$ contingency table. Many cells of the contingency table had expected values of less than five items, so statistical significance of the observed $\varkappa^2$ was evaluated using a randomization test involving 1000 permutations of the data (see Noreen 1989).

Diet composition based on esophagus and stomach contents was related to movement with a polytomous logistic regression (Hosmer and Lemeshow 1989). This type of regression predicts the ln-transformed ratio of the probability of observing one prey category (P_1) divided by the probability of observing another prey category (P_2) (i.e., $\ln(P_1/P_2)$) as a function of one or more independent variables. If there are N prey categories, then N−1 regression equations are calculated. Statistical significance of the overall model, of each independent variable considered, and of the individual variables estimated was evaluated using likelihood ratio and Wald statistics (Hosmer and Lemeshow 1989). We present correlation coefficients (R's) and partial correlation coefficients (partial R's) to indicate the sign and relative strength of the different relationships examined (Harrell 1986). These R's are similar to correlation coefficients from normal regression techniques, but are calculated from log-likelihoods rather than least squares and tend to be smaller in magnitude (SAS Institute Inc. 1989).

With logistic regression the number of items in the least frequent category can constrain the number of independent variables considered (Harrell 1986). Prey types were therefore pooled into three categories: (1) crustaceans (copepods and ostracods), (2) dipteran larvae, and (3) insect pupae and adults (dipteran pupae, and adult dipterans and collembolans). These were expected to approximate benthic, midwater, and surface prey, respectively, based on earlier experience with this system (Grant and Noakes 1986), descriptive accounts of the prey taxa (Pennak 1978; Cooper et al. 1985), and qualitative observations of where stream salmonids cap-

TABLE 2. Partial correlation coefficients between the movement variables and measures of variation in the individual's local environment, body size, and water-column use. $^{**}P < 0.005$.

Source of variation	Movement variable		
	Proportion of time spent moving	Speed while moving	Pursuit distance
Date	−0.13	−0.04	0.05
Time-of-day	−0.03	0.11	−0.07
Water temperature	0.07	0.08	0.18
Pool surface area	0.25**	0.08	−0.04
Water depth	0.33**	0.34**	0.30**
Fork length	−0.05	0.05	0.10
Proportion of feeds at surface	0.51**	0.30**	0.56**
N	218	103	110
R^2	0.41	0.25	0.43

TABLE 3. Partial correlation coefficients for body depth and caudal fin depth after adjusting for standard length and caudal peduncle depth after adjusting for the depth of the caudal fin. All correlation coefficients are nonsignificant ($P > 0.05$.).

Shape variable	Movement variable		
	Proportion of time spent moving	Speed while moving	Pursuit distance
Body Depth	0.06	0.11	0.01
Caudal fin depth	0.01	0.07	0.01
Caudal peduncle depth			
Including fin fold	0.12	−0.03	0.15
Excluding fin fold	0.08	−0.03	−0.11
N	86	43[a]	47[a]

[a]Quantified by observer 1 only.

ture different prey (e.g., Williams 1981; McNicol et al. 1985). After pooling, there were still only 64 items in the insect pupae and adults category, so we have presented the best five-variable regression model here (see Hosmer and Lemeshow 1989).

Prey categories present in the stomach were compared with those present in the intestine as an approximate measure of the longer term repeatability of diet composition. These data were analyzed using a repeated measures design with two repeated measurement factors: prey category (benthic, midwater, and surface) and portion of the digestive tract (stomach and intestine). Log-linear models for contingency tables (Sokal and Rohlf 1981) were used to examine whether the probability of surface prey being present in the stomach was negatively related to the presence of benthic prey.

Results

Movement Patterns and Environmental Variation

The proportion of time spent moving, the speed while moving, and pursuit distance were not strongly correlated with the date or time-of-day when individuals were observed (Table 2). Because changes within a day can be periodic, we added squared and cubed terms for time-of-day to the analyses described in Table 2. These additional terms were all nonsignificant (all P's > 0.50). Similarly, none of the partial correlation coefficients between the movement variables and water temperature was significant (Table 2).

The variation in movement was weakly related to spatial variation in the local environment in which individuals were observed (Table 2). Fish in pools with larger surface areas spent significantly more time moving than those in pools with smaller surface areas, but this trend was weak. For example, the 25 most active fish moved 92–100% of the time and were observed in pools of 0.4–10.4 m^2, while the 25 least active individuals moved 0–1% of the time and were observed in pools of 0.1–10.4 m^2. Significant correlations were not found between pool surface area and the other movement variables. Once other potential sources of variation were controlled, fish foraging in deeper water also spent significantly more time moving, travelled at faster speeds while moving, and had larger pursuit distances than fish in shallower water. After considering the other sources of variation, pool surface area and water depth accounted for an additional 11, 10, and 6% of the variation observed in the proportion of time spent moving, the distance moved per unit time during moving search, and pursuit distance, respectively.

Movement Patterns and Morphological Variation

The captured fish provided a representative sample of the observed variation in behaviour. The mean proportion of

time spent moving was 0.38 (SD = 0.34, range 0–1.0, N = 86 individuals) (compare with Table 1). The mean speed while moving was 2.7 fork lengths ·5 s^{-1} (SD = 1.1, range 1.0–4.9, N = 43), and the mean pursuit distance was 3.0 fork lengths (SD = 0.4, range 0–8.5, N = 47). Fork lengths ranged from 1.9 to 2.9 cm ($\bar{x}$ = 2.3, SD = 0.3).

Larger trout were no more active than smaller ones (proportion of time spent moving versus standard length: r = 0.12, one-tailed $P > 0.10$, N = 86; also see Table 2). Larger trout did travel faster while moving and had larger pursuit distances than smaller trout, even though distances were measured in terms of relative size (fork length) (r = 0.28, one-tailed P = 0.05, N = 42, and r = 0.33, one-tailed $P < 0.01$, N = 47, respectively). However, these trends were not significant once environmental factors were considered (P's > 0.05; also see Table 2).

The proportion of time spent moving was not correlated significantly with three measures of body shape widely thought to influence foraging movements (Table 3). Multiple regression analysis indicated that standard length, body depth, caudal peduncle depth, and caudal fin depth accounted for only 3% of the variation in the proportion of time spent moving (R^2 = 0.03, F = 0.41, $P > 0.80$). The speed while moving and pursuit distance were also not correlated significantly with these measures of shape (Table 3).

Movement Patterns, Water-Column Use, and Diet

Independent of body size and environmental variation, individuals that directed a greater proportion of their foraging attempts at the water surface spent more time moving, travelled at faster speeds while moving, and had larger pursuit distances than individuals foraging away from the water surface (Table 2; Fig. 1). For the proportion of time spent moving, the speed while moving, and pursuit distance, water-column use accounted for an additional 21, 6, and 26% of the variation, respectively, above that already explained by the other variables.

Captured individuals had 0–17 prey items (median = 4) in their esophagus and stomach. Of the six fish that had no prey in their esophagus and stomach, four had prey remains in their intestine, demonstrating that they had fed previously. Stomach contents comprised 39.6% copepods, 34.2% dipteran larvae, 11.0% dipteran pupae, 7.2% ostracods, 3.8% dipteran adults, 1.9% collembolans, 1.5% nematodes, and 0.8% unidentified items. Diet varied significantly among the individual fish (χ^2 = 1134, df = 588, randomization $P < 0.001$).

Individuals that spent more time moving ate higher proportions of dipteran larvae, higher proportions of insect pupae and adults, and lower proportions of crustaceans than did individuals that spent less time moving (Fig. 2; Table 4). Although sample sizes for the two other movement variables were smaller, the association between diet and the speed while moving was qualitatively the same as that observed for the proportion of time spent moving (dipteran larvae versus crustaceans: R = 0.11, G = 6.94, $P < 0.01$, df = 1; insect pupae and adults versus crustaceans: R = 0.12, G = 8.21, $P < 0.005$, df = 1; N = 43 individuals). Pursuit distance was also longer for individuals eating more insect pupae and adults versus crustaceans but was not significantly associated with the proportion of dipteran larvae versus crustaceans (R = 0.13, G = 10.76, $P < 0.001$, df = 1, and $R < 0.01$, G = 1.46, $P > 0.20$, df = 1, respectively; N = 47 individuals). In addition, the association between diet and the proportion of time spent moving remained significant after statistically controlling for standard length, sampling date, and the proportions of midwater and surface foraging attempts, although diet was correlated with these variables as well (Table 4). The association between diet and movement was also not a consequence of variation among pools. On 17 occasions, we captured more than one fish from the same pool on the same day (median interval

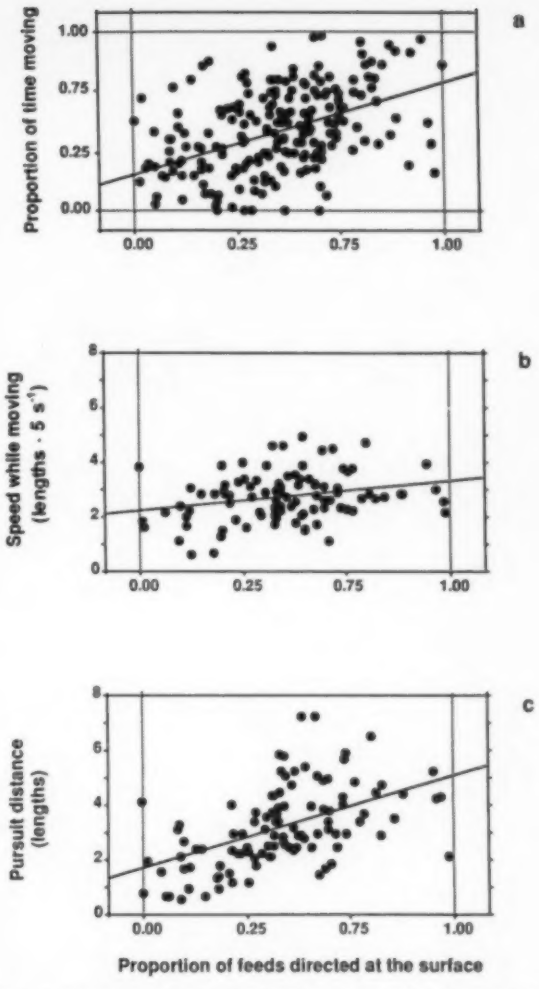

FIG. 1 Leverage plots showing the relationship between the movement variables and the proportion of foraging attempts directed at water surface (PS) after statistically controlling for date, time-of-day, water temperature, pool surface area, water depth, and fork length (see Table 2). Proportions are displayed on an arcsine-square-root scale (see Materials and Methods). Each point represents an individual, and solid lines represent the partial regression equations. (a) Proportion of time spent moving (PM) (regression equation: $\arcsin(PM^{1/2}) = 17.92 + 0.52 \arcsin(PS^{1/2})$, partial r = 0.51, $P < 0.005$, N = 218); (b) speed while moving (SWM) (regression equation: $SWM = 2.2 + 0.01 \cdot \arcsin(PS^{1/2})$, partial r = 0.30, $P < 0.005$, N = 103); (c) pursuit distance (PD) (regression equation: $PD = 1.72 + 0.04 \cdot \arcsin(PS^{1/2})$, partial r = 0.56, $P < 0.005$, N = 110).

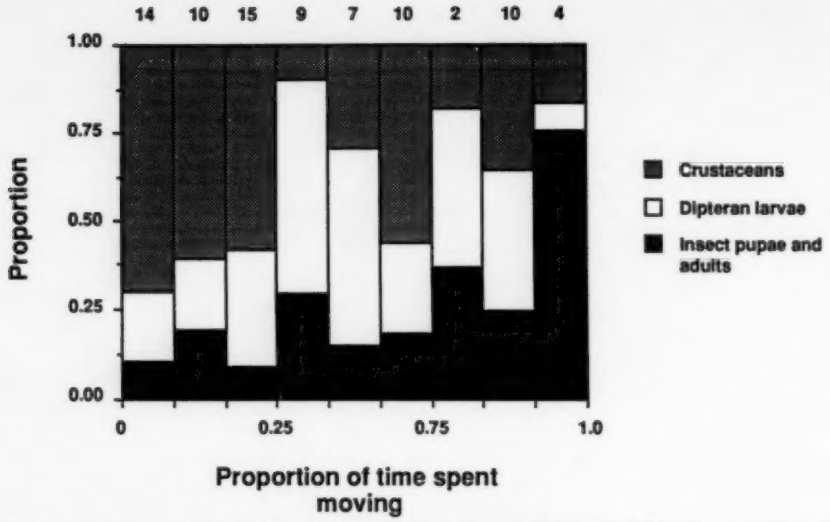

FIG. 2. Diet composition for recently emerged brook trout that spent different proportions of time moving. The proportion of time spent moving is displayed on an arcsine-square-root scale (see Materials and Methods). This scale was divided into nine class intervals. Proportions by number of the prey categories were calculated using the fish within each class interval. Numbers of fish in each interval are presented at the top of the figure. Only fish with food in their stomach and measures of movement were included.

TABLE 4. Partial correlation coefficients from a logistic regression relating stomach contents of individual brook trout to their movement, size, water-column use, and sampling date. $*P < 0.05$; $**P < 0.0001$.

	Relative proportions of prey categories	
Source of variation	Dipteran larvae vs. crustaceans	Insect adults and pupae vs. crustaceans
Proportion of time spent moving	0.11**	0.11**
Standard length	0.06*	0.13**
Date	−0.06*	0.11**
Proportion of feeds at surface	0.04	0.05*
Proportion of feeds in midwater	0.12**	0.06**

between captures = 1.5 h). After adjusting for the variation among samples, fish that spent a greater proportion of time moving had more dipteran larvae, more insect pupae and adults, and fewer crustaceans in their diet than fish from the same pool that spent a lower proportion of time moving (dipteran larvae versus crustaceans: $R = 0.17$, $G = 11.04$ $P < 0.001$, df = 1; insect pupae and adults versus crustaceans: $R = 0.10$, $G = 4.60$, $P < 0.05$, df = 1).

Individuals with a particular prey category present in their stomach tended to have the same prey category present in their intestine (Table 5). With the repeated measures analysis, a model considering only the main effects provided an adequate fit to the observed presence/absence data (residual $\chi^2 = 5.84$, $P > 0.05$, df = 2). More importantly, the numbers of fish that had eaten each prey category were not significantly different across stomach and intestinal samples ($\chi^2 = 0.99$, $P > 0.30$, df = 1).

Individual fish did not completely specialize on one particular prey category (Fig. 3). Forty-seven percent (43/91) of the fish had two prey categories present in their stomach and 12% (11/91) had all three. However, log-linear analysis of the presence/absence data for stomachs revealed a significant, negative interaction between feeding on crustaceans (benthic prey) and feeding on insect pupae and adults (surface prey) (parameter estimate = −0.24; $G = 4.85$, $P < 0.05$, df = 1). The model including this interaction provided an adequate fit of the data ($G = 3.36$, $P > 0.30$, df = 2) and a significantly better fit than a model assuming complete independence among the prey categories ($G = 5.04$, $P < 0.025$, df = 1). Therefore, regardless of whether dipteran larvae were present in the diet, individuals that had eaten a crustacean were less likely to have eaten an insect pupa or adult than individuals that had not eaten a crustacean.

Movement Patterns and Food Intake

All of the trout made foraging attempts during the period of data collection (Fig. 4). Individuals that spent more time moving made more foraging attempts per unit time than

TABLE 5. Prey categories observed in the stomachs and intestines of individual recently emerged brook trout.

Intestinal contents	Stomach contents: Crustaceans		Dipteran larvae		Insect adults and pupae	
	Present	Absent	Present	Absent	Present	Absent
Present	42	11	43	5	32	1
Absent	6	25	13	23	3	48

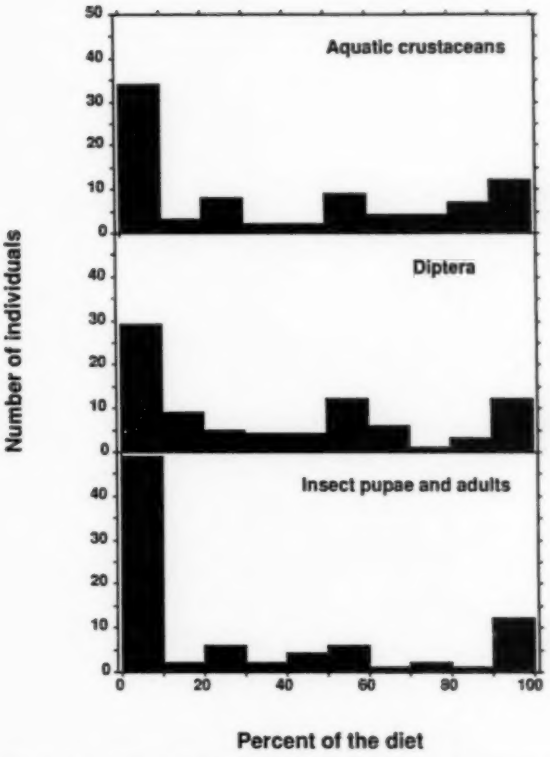

FIG. 3. Number of individual brook trout having a given percentage of each prey category in their diet. (a) Crustaceans (benthic prey); (b) dipteran larvae (midwater prey); (c) insect pupae and adults (surface prey).

those that spent less time moving, independent of other aspects of movement, fork length, and temporal and spatial variation in the local environment (Table 6; Fig. 4). The rate of foraging attempts was also positively correlated with water temperature after stastistically adjusting for the other sources of variation (Table 6).

The number of prey items in the stomach was not significantly correlated with the proportion of time spent moving, the speed while moving, or pursuit distance ($r = 0.04$, $P > 0.70$, $N = 86$; $r = 0.18$, $P > 0.20$, $N = 42$; and $r = 0.07$, $P > 0.60$, $N = 47$, respectively). After adjusting for variation in standard length among the trout, the mean length of prey in stomachs was slightly larger for individuals that spent more time moving than for those that spent less time moving (partial $r = 0.24$, $P < 0.05$, df = 76), but prey length

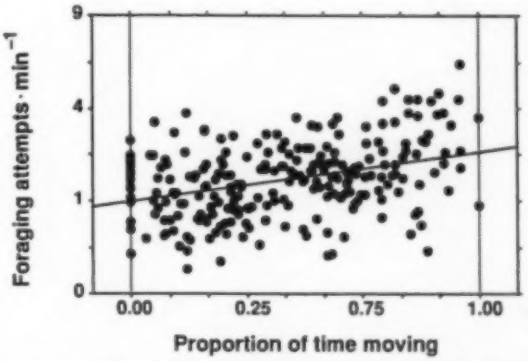

FIG. 4. Feeding rate (FR) versus the proportion of time spent moving (PM). The solid represents the regression equation $FR^{1/2} = 0.99 + 0.006 \cdot \arcsin(PS^{1/2})$, $r = 0.37$, $P < 0.0001$, $N = 251$.

was not significantly related to the speed while moving or pursuit distance (partial $r = 0.13$, $P > 0.40$, df = 37 and partial $r = 0.01$, $P > 0.90$, df = 42, respectively).

Discussion

Our findings support three main conclusions. First, the patterns of foraging movements exhibited by recently emerged brook trout were related most strongly to the vertical location in the water column where fish captured prey, to the types of prey consumed, and to the foraging attempts made per unit time. Second, the patterns of foraging movements were related less strongly to spatial heterogeneity in the locations (pool sizes, water depths) where individuals were observed. In fact, individuals exhibiting different patterns of foraging movements and having different diets were sampled from the same pool on the same day. Third, the patterns of foraging movements were not significantly related to measures of temporal variation in the environment (i.e., date, time-of-day, water temperature), to body size and shape, or to a measure of hunger (amount of food in the stomach).

Our findings suggest that differences in the spatial distribution (benthic, midwater, or surface) and the behaviour of the prey types can facilitate divergent foraging behaviour and diet among the trout. Trout that exhibit long pursuit distances, a high proportion of time spent moving, and fast speeds while moving direct more foraging attempts toward the water surface, eat more insect prey and fewer crustaceans, and make more foraging attempts per minute than trout that exhibit short pursuit distances, a low proportion of time spent moving, and slow speeds while moving. Pursuit

TABLE 6. Partial correlation coefficients relating the number of foraging attempts made per minute to the movement variables, fork length, water temperature, pool surface area, water depth, date, and time-of-day. $^{**}P < 0.001$.

Source of variation	Partial correlation
Proportion of time spent moving	0.36**
Speed while moving	−0.10
Pursuit distance	0.18
Date	0.02
Time-of-day	0.13
Water temperature (°C)	0.27**
Pool surface area (m^2)	−0.01
Water depth (cm)	−0.13
Fork length (cm)	0.00
N	103[a]
R^2	0.33

[a]Only observer 1 quantified all three movement variables.

distances are probably longer when the trout feed on surface prey because surface items are larger (geometric $\bar{x}$ lengths = 3.0 mm) and probably visible at greater distances than midwater and benthic items (geometric $\bar{x}$ lengths = 2.6 and 0.8 mm, respectively). As well, insect adults and pupae may be easier to detect against a well-lit water surface than dipteran larvae in the water column or crustaceans against the pool bottom. The insect prey also appear to be widely dispersed and sedentary; thus, fish increasing their activity levels would increase their encounter rate with this prey type. Conversely, the crustaceans may avoid fish that are moving by swimming into the loose silt at the bottom of a pool. To feed successfully from the benthos, a fish must apparently wait for the crustaceans to move away from cover. Increased detectability and less effective escape abilities of surface prey versus benthic prey could also explain why the rate of foraging attempts is higher for fish that spend a greater proportion of time moving than it is for fish that spend a lower proportion of time moving.

The correlations between movement, water-column use, and diet are probably important in the development and maintenance of the individual variation in diet reported previously for several salmonid species, including brook trout (Bryan and Larkin 1972; Bryan 1973; Allan 1981; McNicol et al. 1985; Ringler 1985). Bryan and Larkin's study concluded that diets of individuals in the field were most similar over several weeks, but some specialization persisted for up to half a year. The differences were maintained among individuals that moved between captures (Bryan and Larkin 1972), and laboratory studies suggested that previous experience with a prey type and the feeding history of an individual's parents were insufficient to account for the variation (Bryan 1973). The differences in diet were thought to reflect slight individual variation in search behaviour and capture technique, and our findings demonstrate that in the field, individual brook trout feeding on different prey types exhibited different search and pursuit techniques. Determining how the variation observed among brook trout arises will require further investigation. However, it probably reflects selective use of different foraging locations rather than active choice for particular prey types per se, as Holbrook and Schmitt (1992) found in striped seaperch (*Embiotoca lateralis*). We often observed trout attacking and rejecting inedible objects in the water column which suggests that they were not specializing on the types of prey they attacked.

Whether the variation in diet and movement observed for recently emerged brook trout is as stable as the dietary specialization reported by Bryan and Larkin (1972) is unclear because the durations of our observation periods were short, and the fish were too small to mark and reobserve in the field. Nevertheless, our analyses furnish some insight into the longer term stability of the variation. We have no reason to believe that the behavioural variation reflects stable, repeatable differences related to alternative genotypes or fixed alternative phenotypes. For example, our analysis of morphology provided no indication that an individual's movement behaviour was related to its body size or shape. In fact, some fish clearly switched between periods of moving and not moving while they were foraging, although most fish tended to specialize at one role over the duration of our observations (McLaughlin et al. 1992). This suggests the variation reflects alternative roles adopted by flexible individuals. However, evidence from digestive tract contents leads us to believe that the variation among individuals persists for periods substantially longer than the duration of our observation periods. For example, stomach contents reflect the feeding history of an individual over the previous 5–6 h and intestinal contents up to 18 h (Miller 1974; Williams 1981). The relationship between the proportion of time spent moving and diet and the similarity between the prey types in the stomach and those in the intestine would not be expected if individuals were changing their foraging behaviour frequently.

Two conditions favouring the development of short-term specializations also appear to be met in our study system. First, prey preference, averaged over all of the trout, appears to be weak and highly variable (Fig. 3). In addition, the probability of a trout having a surface prey present in its diet was lower if benthic prey had been eaten than it was if benthic prey had not been eaten. This suggests the trout could not exploit the different food sources simultaneously, possibly because of travel costs associated with switching between the benthos and the water surface (e.g., Murdoch et al. 1975) or because of learning costs associated with switching between different prey types (e.g., Bence 1986). Second, density-dependent growth and survival are known to occur during early life stages in many stream-dwelling populations of salmonids (Elliott 1989; Grant and Kramer 1990). With a population of individuals foraging on varied but limited resources, and costs for switching between behavioural options, the frequency- and density-dependent processes comprising the social environment can produce relatively stable alternative foraging patterns much like those predicted by models of habitat selection for unequal competitors (Partridge and Green 1985). This outcome contrasts with the single foraging strategy predicted by the conventional optimal foraging models which do not account for an individual's social environment (Stephens and Krebs 1986). Earlier studies of movement patterns proposed that variation in predator mobility could facilitate resource partitioning among species, thereby allowing potential competitors to coexist (Gerritsen and Strickler 1977; Huey and Pianka 1981; Paszkowski 1985; Johansson 1991). Such a mechanism may be operating at the individual level in brook trout.

More active trout foraged in deeper water, on average, than less active trout. This relationship reflects, in part,

a tendency for sedentary individuals to be close to the stream bank, possibly because the abundance of crustacean prey was higher there than in more open water (e.g., Castonguay and FitzGerald 1991) or because predation risk was higher for individuals near the bank versus those further away. It probably also reflects the influence of pool morphometry because trout in pools with smaller surface areas also spent a lower proportion of time moving than those in larger pools. However, it is the proportion of sedentary versus active fish that varied across pools of different surface area; both types of fish were observed across virtually the entire range of pool sizes (see Results), and both types of fish were often observed in the same pool at the same time (McLaughlin et al. 1992). This is the sort of outcome expected if frequency- and density-dependent processes were operating to favour alternative behavioural roles, and if pool morphometry was influencing the relative abundances of benthic and open-water prey and hence the number of individuals adopting each role (Murdoch et al. 1975). It is not the outcome expected if pool morphometry was limiting activity or influencing the level of activity maximizing an individual's net rate of energy gain.

Earlier comparative studies successfully related interspecific variation in foraging movements to measures of locomotor ability (e.g., sprint speed, endurance) (Huey et al. 1984) or to physiological (e.g., anaerobic and aerobic scope) and morphological (body size and shape, muscle morphology) attributes expected to influence locomotor ability (see the introduction). If these phenotypic attributes influence foraging movements, then it is reasonable to expect that they may be correlated with movement at the level of individuals (Bennett 1987). Studies of foraging patterns conducted at the intraspecific level, however, have often emphasized the notion of a single phenotype with flexible behaviour, rather than alternative phenotypes with restricted behaviour (Helfman 1990), and important phenotypic effects on movement have been overlooked even in well-studied species (Ehlinger and Wilson 1988; Ehlinger 1990; Kieffer and Colgan 1991). Our study is one of few attempts to statistically relate quantitative field measurements of foraging movements to phenotypic and environmental factors expected to influence an individual's swimming performance. Body morphology could either limit an individual's swimming capacity or affect the level of activity that would maximize an individual's net rate of energy gain. Similarly, water temperature can influence a fish's swimming capacity, and the speed that would maximize the fish's net rate of energy gain. Our analyses provided no evidence that either factor accounted for the variation observed among the trout in these still-water pools.

Small sample sizes can impede attempts to reveal correlations between morphology and locomotor behaviour at the individual level (Arnold and Bennett 1988). Our sample was large enough to detect significant correlation coefficients as low as 0.18, and a correlation coefficient of 0.30 would have been detected with probability 0.88. In addition, the trout were observed at an early stage of their life history leaving only a short period where postnatal selection could have eliminated the behavioural or morphological extremes (Arnold and Bennett 1988). Finally, the negative findings reported here for body size and shape are not a consequence of the short duration of our observations or of the morphological attributes we considered; using the same methods, we did detect significant differences in standard length, body depth, caudal peduncle depth, and caudal fin depth among individuals of similar size foraging in locations differing from 0 to 12 $cm \cdot s^{-1}$ in current velocity (McLaughlin and Grant 1994). The pattern observed across running- and still-water habitats versus that reported here for trout exhibiting different patterns of foraging movements within still-water habitats probably occurs because trout in running water swim more continuously and at faster speeds than do even the most active trout in still-water pools (McLaughlin and Grant 1994).

Our comparison of individual trout has been valuable for identifying phenotypic and environmental factors expected to influence patterns of foraging movements and evaluating their relative contribution to the broad variation in activity exhibited by trout in the field. Although we have not linked the individual variation in foraging movements directly to variation in growth or survival, the correlations between movement, prey type, and the rate of foraging attempts do suggest that the variation has significant ecological implications because growth rate can depend on the type of food consumed as well as the amount (Brett 1979). In addition, recent theoretical and empirical studies have provided ample reason to believe that such individual variation in foraging behaviour during early life stages can have significant implications for population dynamics (Elliott 1989, 1990a, 1990b; DeAngelis and Rose 1992). Measuring the longer term consequences for trout using different patterns of foraging movements presents a challenging and important problem for future investigation.

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Assessment of the Estuarine Hermaphroditic Fish *Rivulus marmoratus* as a Useful Euryhaline Species for Acute Toxicity Tests as Shown Using Cadmium

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The estuarine hermaphroditic teleost *Rivulus marmoratus* was sensitive to toxic effects of cadmium showing low 96-h LC50 values of 0.8–32.8 mg Cd/L for different life stages at 10‰ salinity, 25°C, and pH 7.0. Toxicity was greater at higher temperatures, at higher pH, and at lower salinity. Tolerance to cadmium increased after pretreatment with a low dose of cadmium, but the effect decreased when intervals between pretreatment and challenge treatment were longer than 4 d. The gill lamellae and kidney tubules were the primary target organs for acute toxic effects of cadmium in adult fish. These results suggest that *R. marmoratus*, which is small, physiologically tolerant, hardy, and easy to breed, may prove to be a useful euryhaline model for estuarine metal toxicity studies.

Le téléostéen hermaphrodite estuarien *Rivulus marmoratus* était sensible aux effets toxiques du cadmium et présentait des valeurs faibles de CL_{50}-96 h de 0,8 à 32,8 mg Cd/L à différents stades de vie pour une salinité de 10 ‰, une température de 25 °C et un pH de 7,0. La toxicité était plus grande pour des températures élevées, un pH plus élevé et une salinité plus faible. La tolérance au cadmium augmentait après un prétraitement au cadmium à faible dose, mais les effets diminuaient lorsque l'intervalle entre le prétraitement et le traitement dépassait quatre jours. Les effets toxiques aigus du cadmium chez le poisson adulte se manifestaient principalement au niveau des lamelles branchiales et des tubules rénaux. Ces résultats semblent indiquer que *R. marmoratus* qui est petit, physiologiquement tolérant, robuste et qui se reproduit facilement, peut se révéler un modèle euryhalin utile pour des études de toxicité des métaux en milieu estuarien.

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Cadmium is regarded as one of the most toxic heavy metals, and the potential for fish exposure has increased with increasing industrial use of this metal. While a large number of reports on cadmium toxicity to freshwater fish has been accumulated from diverse species for past two decades (Sprague 1987), much less information is available regarding the toxicity of this metal to marine and estuarine fish. Studies of estuarine species are particularly limited (McLeese et al. 1987). Estuaries are important for both freshwater and marine fish, since they are used for breeding and feeding. Catadromous and anadromous fish move through estuaries during migration and may stay in them for some time. Recently, adverse impacts of fish habitats in estuaries could result from a steady decline in water quality associated with human population growth and industrialization of coastal areas (Cross et al. 1985). Thus the effect of cadmium on estuarine fish is of interest for assessing metal toxicity in these dynamic ecosystems.

The oviparous rivulid fish *Rivulus marmoratus* (synonym *R. ocellatus marmoratus*) inhabits estuaries throughout the Caribbean and is the only known vertebrate that exhibits natural functional hermaphroditism, with internal self-fertilization (Harrington 1961). Consequently, a given group of individuals originating from one progenitor is genetically homogeneous (Kallman and Harrington 1964; Vrijenhock 1985). This is a useful feature for excluding the effects of differing genetic makeup from toxicity studies. This fish has other desirable attributes: it has an extremely broad physiological tolerance, being euryhaline (freshwater to 35‰ salinity) and eurythermal (4–40°C), which allows study of salinity and temperature as factors modifying toxicity. The fish is well adapted for low oxygen, since it can carry out aerial respiration through the skin (Grizzle and Thiyagarajah 1987). The fish are small (3–5 cm), hardy, and easy to breed

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in captivity, making for a useful test species for estuarial toxicity studies. Therefore, for development of *R. marmoratus* as an animal model for the study of heavy metal toxicity to estuarine biota, cadmium toxicity was evaluated as a vehicle for assessing this fish as a model.

Materials and Methods

Rivulus was bred and reared in aquaria as described previously (Park and Kim 1984; Park and Lee 1988). Our fish stock originated from a single individual obtained from the Zoology Institute and Museum, University of Hamburg, in August 1981. Fish were housed in groups in 40-L glass tanks containing aerated water of 10 ± 0.5‰ salinity. They were kept in an air-conditioned room at 25 ± 1°C with a daily cycle of 14 h light : 10 h dark. Fish less than 1 mo old were fed brine shrimp nauplii (*Artemia salina* eggs from Metaframe, California), and adults were nourished with chicken liver and fruit flies (*Drosophila*).

Static toxicity tests were conducted for larval (7 ± 2 d old), juvenile (30 ± 2 d old), and adult (120 ± 2 d old) stages. On the day prior to a test, 0.5 L of water at 10‰ salinity was placed in a 1-L transparent plastic bottle. Water pH was adjusted to 7.0 with H_2SO_4. The test water was saturated with oxygen by continuous aeration prior to loading the fish. Fifty healthy larvae were selected and placed in each test bottle containing $CdCl_2$ (analytical reagent grade, Merck, Germany) of the appropriate test concentration. Cadmium concentrations in the tests were measured using an atomic absorption spectrophotometer (Perkin-Elmer 2380, U.S.A.). The measured concentrations, however, varied from nominal concentration within 4%. The background cadmium levels were not detected in the test water. For juvenile and adult fish, 20-L plastic containers filled with 10 L of test water were used. Loading densities were maintained at 5 juveniles/L and 1 adult/L. Aliquots of the cadmium stock solution were added to the test containers just before animals were introduced to obtain the desired concentrations. The test containers were completely sealed and kept in an incubator at 25 ± 0.1°C for 96 h. The fish were starved for 1 d prior to and during tests. The criteria for death were lack of opercular movement and heartbeat and response to mechanical stimulus over a period of 2 min.

To evaluate the effect of salinity, fish larvae were held for 96 h at 1, 10, 20, or 30‰ salinity (approximately 2.9, 29, 58, and 87% seawater) containing 0.8 mg Cd/L at 25°C. Duplicates with 100 fish each were tested. The effect of temperature was assessed by exposure of larvae for 96 h to 0.8 mg Cd/L at 20, 25, and 30 ± 0.1°C. The effect of pH on toxicity was assessed by exposing 50 larvae for 96 h in 0.5 L of test solution at 0.8 mg Cd/L for various levels of pH from 6.0 to 8.0. A preliminary experiment showed no pH toxicity to *Rivulus* larvae in those ranges. Duplicate exposures were carried out at each pH.

The effect of cadmium pretreatment was tested by exposing five groups of juvenile fish to 5 mg Cd/L for 24 h. One to 4 d after that pretreatment, they were challenged by a lethal concentration (25 mg/L) of cadmium at 25°C for 96 h. Duplicate exposures with 60 fish in each group were used.

Histological observations were made on adult fish (3.0–3.5 cm total length) exposed to 35 mg Cd/L for 96 h and sampled at 1, 2, 3, and 4 d following that exposure. Fish were anesthetized in ice-cold water and internal organs were exposed by dissecting the belly. Whole fish were then fixed with 10% neutral buffered formalin for 2 d. They were then dehydrated in a graded ethanol series and embedded in paraplast. Every embedded whole sample was sectioned from mouth to anus at 5 μm and then stained with hematoxylin and eosin.

The 96-h LC50s and their 95% confidence intervals (CI) were calculated by probit analysis using Labcat Module Package, Version 4.0 (Innovative Programming Association, Princeton). Significant differences were assessed by *t*-tests at a significance level of $p < 0.05$.

TABLE 1. Comparison of 96-h LC50 cadmium concentrations and 95% CIs for different life stages of *R. marmoratus* at 25°C, pH 7.0, and 10‰ salinity.

Life stage	Age (d)[a]	LC50 (mg/L)	95% CI (mg/L)
Larva	7 ± 2	0.8	0.4 – 1.4
Juvenile	30 ± 2	18.8	18.2 – 19.2
Adult	120 ± 2	32.2	30.3 – 33.7

[a]Posthatching.

Results

The younger life stages were more sensitive to cadmium than the older stages (Table 1). Larvae were nearly 40 times more susceptible to cadmium than adult fish.

Higher temperature and lower salinity induced greater toxicity of cadmium to fish (Fig. 1a and 1b). Mortality increased approximately 30 times as salinity dropped from 30 to 10‰. At 30°C the metal was approximately 20 times as toxic as at 20°C. The influence of pH was also pronounced even in the range of pH 6.0–8.0 (Fig. 1c). Lethality was nearly five times greater at pH 8.0 than at pH 6.0.

Cadmium-pretreated fish were significantly more resistant than control fish, within 3 d of pretreatment (Fig. 2). No pretreatment effects were observed, however, when the challenge was 4 d later than pretreatment.

The gross and histologic appearances of gills of adult *Rivulus* were essentially similar to those of other marine teleosts (Fig. 3a). Exposure to 35 mg Cd/L resulted in swollen gill lamella within 24 h, and a breakdown of gill epithelium was evident by 48 h (Fig. 3b). The lamellae of the gill filaments were completely destroyed at the final stage of death. Besides the breakdown of respiratory epithelium, chloride cells were also destroyed and could not be found in the gill filaments.

The kidney of control *Rivulus* is composed of much nephrous and interstitial lymphoid tissue, as in other teleosts (Fig. 4a). A considerable degree of histopathological change appeared in cadmium-treated fish. Cloudy swellings of renal tubules were noted within 24 h after cadmium exposure. The affected cells underwent hypertrophy and degenerated by 48 h after cadmium treatment. Severe tubular atrophy was observed at the late stage of death (Fig. 4b). Kidney interstitial tissue of fish, unlike that in higher vertebrates, is the major hematopoietic tissue in the body. Due to the similarity in size, shape, and characteristics of hematopoietic and mononuclear inflammatory cells, it was difficult to distinguish the degree of inflammation in the cadmium-treated kidney.

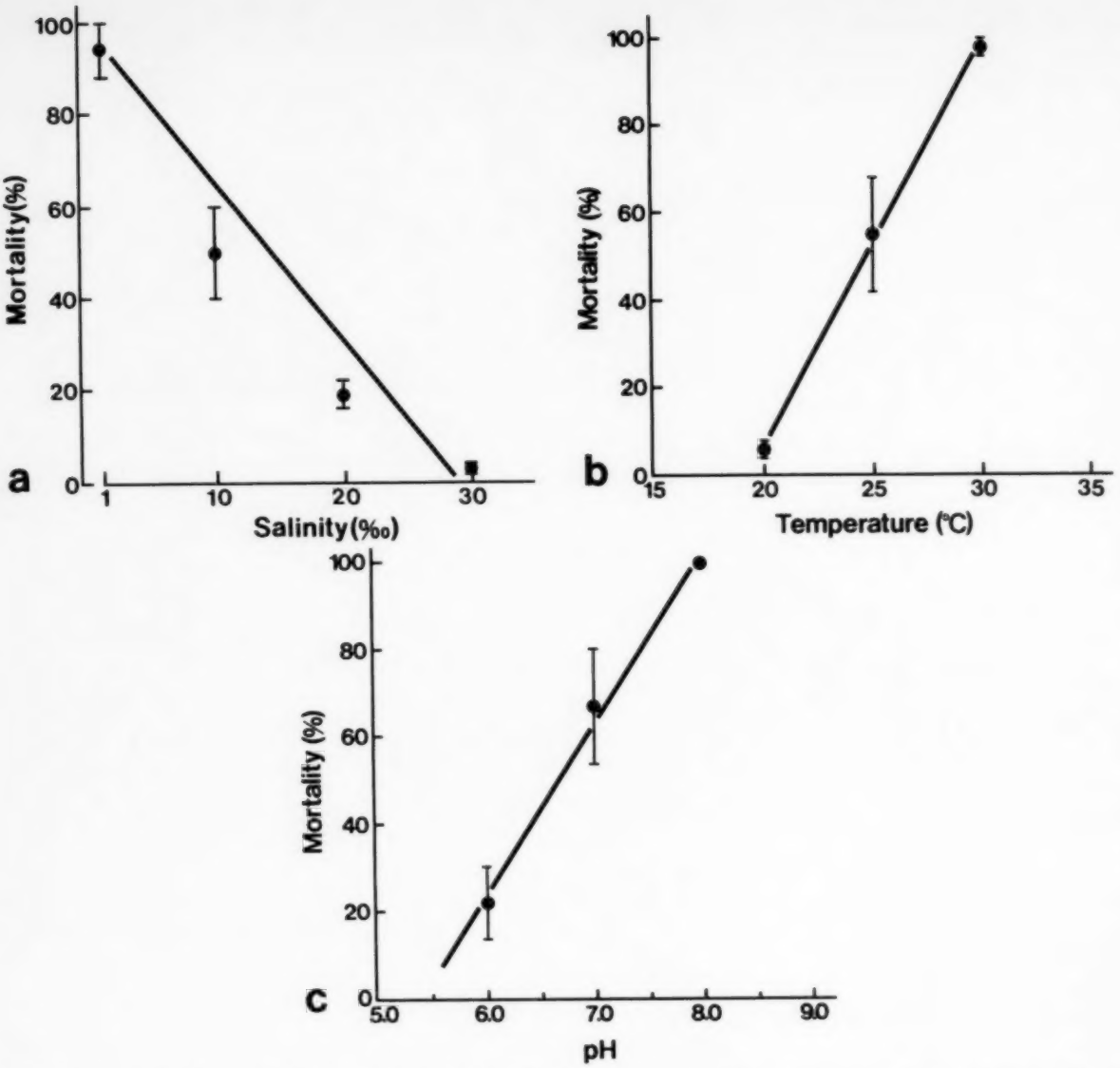

FIG. 1. Effect of (a) salinity (25°C, pH 7.0) ($Y = -3.3X + 93.7$), (b) temperature (10‰, pH 7.0) ($Y = 9.2X - 177$), and (c) pH (25°C, 10‰)($Y = -38.7X - 208$) on acute lethality of 0.8 mg Cd/L to *R. marmoratus* larvae.

TABLE 2. Review of acute toxicity of cadmium to estuarine fish arranged in order of resistance.

Species	Life stage	Temperature (°C)	Salinity (‰)	LC50 (mg/L) 48 h	LC50 (mg/L) 96 h	Reference
Rivulus marmoratus	Larva	25	10	—	0.8	Present study
	Juvenile	25	10	—	19.0	Present study
	Adult	25	10	—	32.2	Present study
Menidia menidia	Adult	20	20	13	—	Middaugh and Dean 1977
Fundulus majalis	Juvenile	20	20	—	21	Eisler 1971
F. heteroclitus	Juvenile	20	20	—	55	Eisler 1971
	Adult	20	20	60	—	Middaugh and Dean 1977

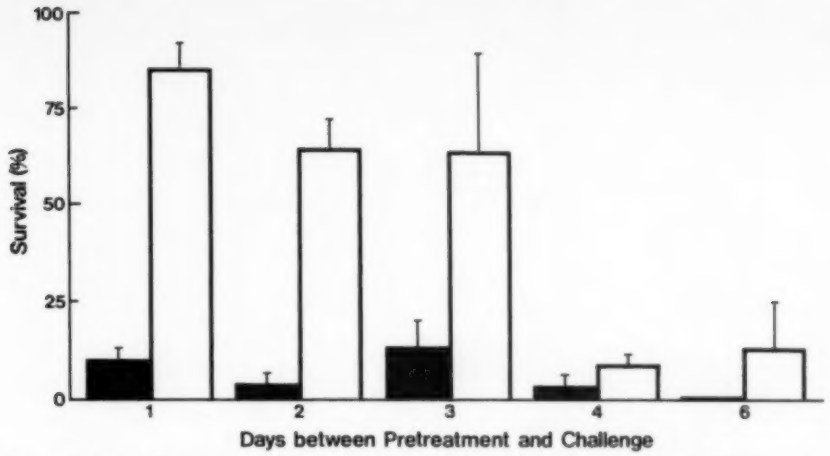

FIG. 2. Effect of pretreatment with a nonlethal concentration (5 mg/L) of cadmium on the acute lethality of challenge treatment (25 mg/L) of cadmium to *R. marmoratus* juveniles at 25°C, pH 7.0, and 10‰ salinity (solid bars = challenge only; open bars = pretreatment plus challenge).

No histopathologic changes were observed in the liver, ovotestis, or alimentary canal.

Discussion

Although fish collected from wild populations are used for toxicity tests, laboratory-cultured fish are more suitable because physiological stress before testing is minimized and because all life stages can be made available. Many laboratory or hatchery stocks of freshwater species such as rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*), goldfish (*Carassius auratus*), Japanese medaka (*Oryzias latipes*), and channel catfish (*Ictalurus punctatus*) are available which are commonly used in aquatic toxicity studies. Laboratory-bred estuarine species are, however, particularly limited. Laboratory stocks of the mummichog (*Fundulus heteroclitus*) are used for test fish, but these fish are not easily bred in aquaria (Atz 1986) and are less susceptible to cadmium poisoning than *Rivulus* (Eisler 1971). *Rivulus* appears to be a relatively sensitive species. It is more susceptible to cadmium poisoning than other estuarine fish judging by a review of previous studies (Table 2). For example, *Rivulus* juveniles have a 96-h LC50 of 18.8 mg Cd/L, while the value for juvenile of mummichog is 55 mg Cd/L even at low temperature and high salinity (Eisler 1971).

In nature, *Rivulus* inhabits mangrove estuaries with low water levels where periodic drying occurs. Therefore, it is well adapted in static, shallow-water conditions where salinity fluctuations are extreme (Davis et al. 1990). This is an additional advantage of this fish for estuarial toxicity.

The toxicity of cadmium to *Rivulus* is greater under conditions of low salinity or high temperature, the phenomena also reported for crabs (O'Hara 1973; Sullivan 1977), isopods (Jones 1975), and mummichog (Eisler 1971). One of the primary factors that modifies cadmium toxicity to estuarine organisms seems to be salinity itself, not the change in proportion of free to total cadmium at the various salinities (McLeese et al. 1987). Temperature dependency of cadmium toxicity is probably due to a greater metabolic activity

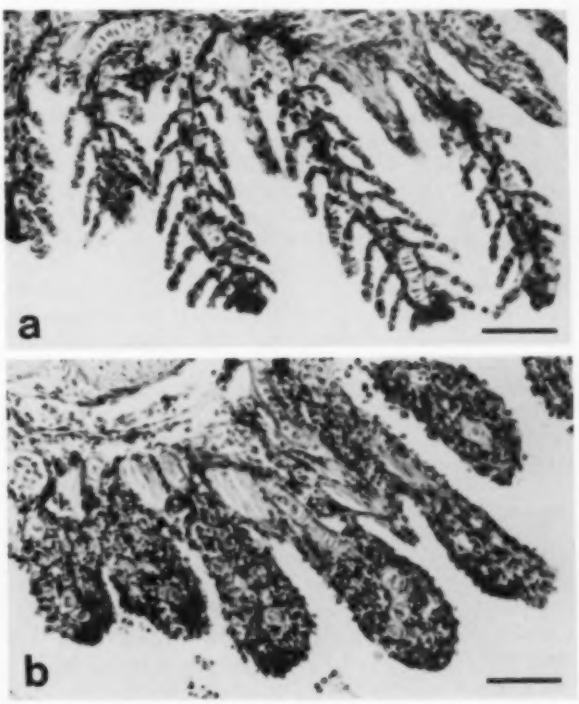

FIG. 3. Gill of adult *Rivulus* treated with cadmium. (a) Gill filament of control fish; (b) gill filament of fish treated with 35 mg Cd/L. H & E stain; bars = 50 μm.

at high temperature which results in an increase in the rate of cadmium uptake. Cellular uptake of cadmium is also highly temperature dependent in mammals (Foulkes 1989).

The effect of pH on acute toxicity of cadmium is not well known for fish, particularly estuarine fish. Freshwater fish show reduced toxicity at higher pH (Pickering and Gast 1972), contrasting with our results for reasons that are

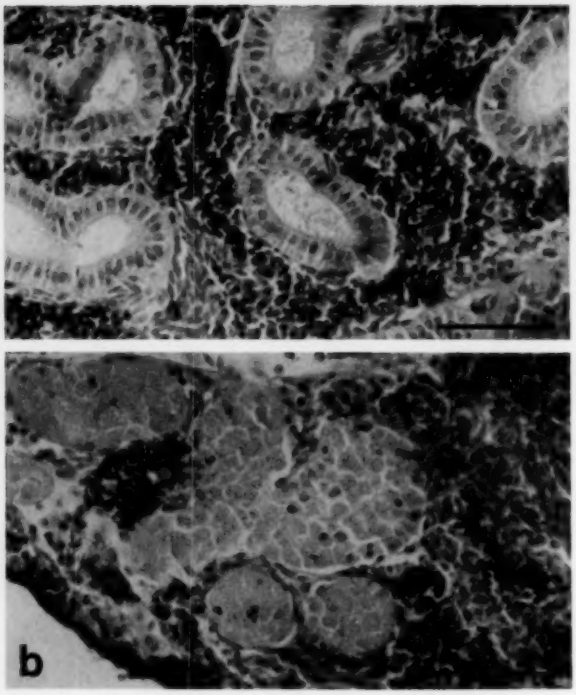

FIG. 4. Kidney of 4-mo-old *Rivulus* treated with cadmium. (a) Kidney of control fish; (b) kidney of fish treated with 35 mg Cd/L. H & E stain; bars = 50 μm.

unclear. Pikering and Gast (1972) suggested that pH-dependent solubility of cadmium might be one of the reasons for differential toxicity.

There are few studies exhibiting increased resistance to cadmium following pretreatment with cadmium, although there are numerous reports on this phenomenon in rodents. Increased resistance to cadmium in pretreated freshwater fish has been reported in rainbow trout (Pascoe and Beattie 1979; Dixon and Sprague 1981), common carp (*Cyprinus carpio*) (Kito et al. 1982), white sucker (*Catostomus commersoni*) (Duncan and Klaverkamp 1983), and fathead minnow (Benson and Birge 1985). These studies suggested that sequestrations of cadmium by cadmium-binding proteins may be involved in this resistance. Thomas et al. (1983a, 1983b) found that rainbow trout removed low levels of cadmium by means of low molecular weight nonmethallothionein proteins. In contrast, at high doses the metal was mainly sequestered by methallothioneins. Hence, resistance observed in our studies may have resulted from detoxification by methallothioneins.

Fish absorb calcium ions through their gills to maintain homeostasis of body fluids (Roch and Maly 1979). Impairment of calcium homeostasis could result from damage to gill tissue. Destruction of the reabsorptive ability of the kidney tubules could also be responsible for a breakdown of calcium ion balance (McLeese et al. 1987). The present study has shown that cadmium damages gill and kidney tissues. This metal has also been reported to damage the gills and kidneys of other estuarine teleosts (Gardner and Yevich 1970; Gardner 1975) and freshwater fish (Oronsaye 1989). While the liver and testis are primary targets of cadmium following acute exposure in mammals (Meek 1959; Hoffman et al. 1975; Dudley et al. 1982), the liver of *Rivulus* is very resistant to the metal. Tafanelli and Summerfelt (1975) also reported greater tolerance of goldfish liver to cadmium. Available evidence indicates that the difference in tolerance between tissues to cadmium toxicity is related to the cadmium-binding capacity of the tissues (Sendelbach and Klaassen 1988). Thus, a possibility of tissue differences in cadmium-binding capacity could not be excluded to explain tolerance of fish liver to cadmium.

Previous studies have shown that *Rivulus* is a useful species for the study of carcinogenesis (Koenig and Chasar 1984; Park and Kim 1984; Thiyagarajah and Grizzle 1986; Park et al. 1993), mutagenesis (Park and Yi 1989), and teratogenesis (Park et al. 1992). Therefore, this fish can serve as a useful animal model for toxicity of cadmium in relation to mutagenesis, carcinogenesis, and teratogenesis.

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Estimation of Recent Growth of Field-Caught Postlarval American Lobsters, *Homarus americanus*, from RNA:DNA Ratios

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Juinio, M.A.R., and J.S. Cobb. 1994. Estimation of recent growth of field-caught postlarval American lobsters, *Homarus americanus*, from RNA:DNA ratios. Can. J. Fish. Aquat. Sci. 51: 286–294.

We developed a growth model for the postlarvae of the American lobster, *Homarus americanus*, using the relationship of RNA:DNA ratios, temperature, and growth rates during postmolt through early premolt of laboratory-reared postlarval lobsters. The model was used to estimate individual growth rates of 385 postlarval lobsters in molt stages C and D_0, collected at two sites in Block Island Sound over three years, 1988–90. The mean growth rates of postlarvae collected in June 1989 and 1990 (0.57 ± 0.19 and 0.54 ± 0.18 mg protein·d^{-1}) were significantly higher than those collected in July (0.39 ± 0.17 and 0.43 ± 0.18 mg protein·d^{-1}). Changes in sea surface temperatures of zooplankton biomass did not account for the seasonal difference in growth rates, nor was there correlation between the biweekly mean growth rates and postlarval densities. The incidence of poorly nourished postlarval lobsters (individuals with growth rates <0.22 mg protein·d^{-1}) was less than 10% of the total samples in each year. We found no evidence that food limitation, resulting in starvation or prolonged duration of the postlarval instar, was a significant factor contributing to the observed interannual variability of postlarval densities.

Nous avons élaboré un modèle de la croissance des postlarves du homard américain *Homarus americanus*, en nous servant de la relation des rapports ARN : ADN, de la température et des taux de croissance entre la postmue et le début de la prémue chez des postlarves de homards élevés en laboratoire. Le modèle a servi à estimer les taux individuels de croissance de 385 postlarves se trouvant aux stades de mue C et D_0, prélevées à deux sites du bassin de l'île Block sur trois années, de 1988 à 1990. Les taux moyens de croissance des postlarves recueillies en juin 1989 et 1990 (0,57 ± 0,19 et 0,54 ± 0,18 mg protéines·$jour^{-1}$) étaient nettement supérieurs à ceux des postlarves prélevées en juillet (0,39 ± 0,17 et 0,43 ± 0,18 mg protéines·$jour^{-1}$). Les changements dans la température superficielle de la mer ou la biomasse zooplanctonique n'expliquaient pas la différence saisonnière des taux de croissance, et on n'observait pas de corrélation entre les taux de croissance moyens bihebdomadaires et les densités des postlarves. L'incidence de postlarves mal nourries (individus dont le taux de croissance était inférieur à 0,22 mg protéines·$jour^{-1}$) était inférieure à 10 % du total des échantillons de chaque année. Nous n'avons trouvé aucun élément indiquant qu'une limitation de la nourriture, causant l'inanition ou prolongeant la durée de l'instar postlarvaire, serait un facteur important agissant sur la variabilité interannuelle observée des densités de postlarves.

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Mortality rates during the postlarval and early benthic stages of the American lobster, *Homarus americanus*, may be critical to determining recruitment strength in any given year (Fogarty and Idoine 1986; Wahle and Steneck 1991). Interannual variability in postlarval abundance has been documented for several locations in the United States and Canada (Scarratt 1973; Fogarty 1983; J.S. Cobb, unpublished data) but the mechanisms contributing to the observed variability in postlarval recruitment are not well understood. Previous studies focused on how hydrodynamic processes and swimming behavior may affect larval and postlarval distribution dynamics (Botero and Atema 1982; Harding et al. 1983; Ennis 1986; Cobb et al. 1989a, 1989b). However, food limitation, which has been alluded to as a major source of larval mortality in lobsters (Harding et al. 1983; Castell and Kean 1986), has received little attention. In the laboratory, food availability and temperature are the major factors affecting the development, growth, and survival of larval *H. americanus* (e.g., Templeman 1936; Carlberg and Van Olst 1976; MacKenzie 1988; Juinio 1991). Similarly, *Pandalus borealis* larvae showed correlations between laboratory feeding regime and survival and growth rates (Ouellet et al. 1992). These findings support the inference that poor larval nutrition may be an important source of larval mortality. However, verification in the field has been lacking, primarily because of the difficulty of measuring nutritional status or growth rates of wild larvae.

In the laboratory, we found that the ratio between RNA and DNA, together with temperature, can be used as an indicator of relative nutritional condition during the intermolt and early premolt periods of the postlarval instar of *H. americanus* (Juinio 1991; Juinio et al. 1992). Moreover, RNA:DNA ratio is a sensitive indicator of recent (2–3 d) food deprivation. This fine time resolution is valuable in inferring short-term changes in prey availability in the field (Buckley

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and Lough 1987). In this paper, we use data from our laboratory experiments on the effects of food availability and temperature on growth rates of postlarvae to develop a growth equation. We then use the equation to estimate the recent growth of individual wild postlarval lobsters in molt stages C and D_0, captured in Block Island Sound. The results allow us to test predictions stemming from the hypothesis that food limitation is a significant factor affecting the observed variability in postlarval recruitment in Block Island Sound during the summers of 1988–90.

We predicted that if food is limiting, (1) a significant number of the postlarvae caught from the field would have lower estimated growth rates than would postlarvae reared under ad libitum feeding conditions in the laboratory and (2) assuming that the postlarvae remained in the nearshore waters, the estimated growth rates of field-caught postlarvae would be higher during, or within a few days after, periods of peak zooplankton abundance. In addition, (3) if survival is dependent on growth rate, a positive correlation between the means of the estimated individual growth rates and total postlarval density in the study area would be expected.

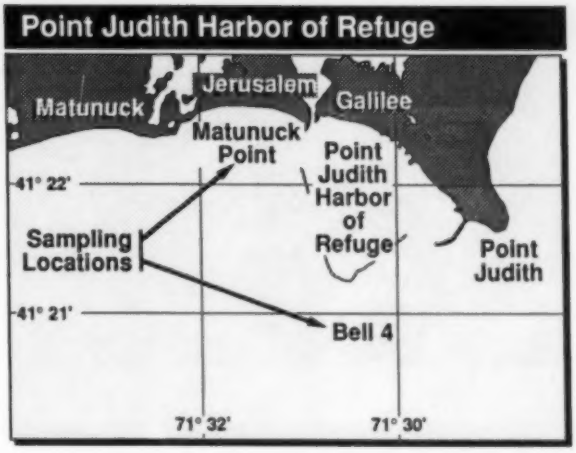

FIG. 1. Sampling sites for postlarval lobsters in Block Island Sound, near Point Judith, Rhode Island, during 1988–90.

Methods and Materials

Growth Model

Details of the laboratory experimentation are reported in Juinio et al. (1992). Briefly, lobster postlarvae (stage IV), obtained from the Environmental Protection Agency Laboratory in Narragansett, Rhode Island, were reared individually in three replicate batches under three different feeding regimes, full ration, low ration, and starved, and three temperature treatments, 15, 18, and 21°C. Full-ration animals were fed San Francisco brand frozen adult brine shrimp ad libitum twice daily, while the low-ration animals received four brine shrimp daily. In addition, these postlarvae were given a lipid supplement (Sasaki 1984) every other day. Individuals in the low-ration treatment were given only four whole adult brine shrimp daily, less than the reported daily requirement for maximal growth in the laboratory (eight adult brine shrimp per day, Carlberg and Van Olst 1976). Starved postlarvae were not provided with food for the duration of the experiment. Each postlarva in the experiment was monitored and mortalities in each treatment were recorded daily.

At least one postlarva from each batch was sampled randomly each day from each treatment tray. The molt stage of each postlarva was determined (according to Sasaki 1984) prior to storage in an ultralow freezer at −80°C. Subsequently, samples were freeze-dried for 24 h and dry weights were determined using a Mettler analytical balance (±0.01 mg). The total RNA and DNA (milligrams per individual) were determined following the modified Schmidt–Thannhauser method used by Buckley (1979) for larval fish. This method was easily adapted for individual lobster postlarvae (Juinio 1991). Total protein for each postlarva was determined colorimetrically using the Sigma bicinchoninic acid protein assay kit with duplicate 100-μL aliquots of the same homogenate used for nucleic acid determinations.

Growth rates (milligrams of protein per day) were determined from the differences in the mean total protein at the start of the experiment and the mean total protein of postlarvae upon attaining stages C and D_0 divided by the intervening number of days for each batch. Since the molt cycle in starved postlarvae was arrested at molt stage C (Juinio et al. 1992), the growth rates for the starved treatments were based on differences in the mean total protein at the start of the experiment and at day 6 for the 18°C treatment and at day 4 for the 21°C treatment. These days correspond to the mean days at molt stages C and D_0, respectively, in the fed treatments reared at the same temperature regimes. Student's *t*-test was used to determine if the mean growth rates attained at molt stage C in the six treatments were significantly different from those attained at molt stage D_0. One-way analysis of variance (ANOVA) was used to compare average growth rates among the six treatments. If a significant difference was found, the ANOVA was followed by Scheffe's *F*-test ($p < 0.05$). to determine which means were significantly different. A growth model using RNA:DNA and temperature for the three temperature regimes and another using only data from 18 and 21°C as predictors of growth rates (milligrams of protein per day) are described.

Field Collections

Postlarval lobsters were collected from neuston tows made at two sites approximately 2 km apart in Block Island Sound near Point Judith, Rhode Island (Fig. 1). Samples were taken two or three times a week from early June through early August during 1988–90. A pair of neuston nets (0.5 × 1 m mouth opening, 500-μm mesh, equipped with mechanical flowmeters) were towed for 10 min at approximately 0.75 $m \cdot s^{-1}$ from a 7-m boat. The volume of water filtered ranged from 165 to 200 m^3 for each net. Two pairs of tows were made at each site on each sampling date. Surface sea temperature was recorded for all tows. All postlarvae were removed immediately from each tow sample and held in chilled seawater until returned to the laboratory, where molt stage was determined. Each postlarva was placed in a separate, labeled vial and frozen at −80°C. Total RNA and DNA were determined with the same methods used for the laboratory-reared animals. The growth rates of field-caught postlarval lobsters in molt stages C and D_0 were estimated using the growth model developed from the laboratory data. The total biweekly density of postlarval lobsters was estimated from

TABLE 1. Average total growth rates (mg protein·d^{-1}), RNA:DNA ratios, and total dry weight (DWt, mg) of lobster postlarvae at molt stages C and D_0 and average temperature (°C) in each treatment. Mean growth rates followed by the same letter are not significantly different (Scheffe's F-test, $p < 0.05$). n = replicate means; SD given in parentheses).

Treatment	n	Growth rate	RNA:DNA	DWt	Temperature
15°C					
Full ration	3	0.167 a	3.32	11.0	15.0
		(0.022)	(0.15)	(0.5)	(0.8)
Low ration	3	0.087 b	3.04	9.7	15.1
		(0.048)	(0.17)	(0.5)	(0.6)
Starved	3	−0.071 c	1.92	5.6	15.0
		(0.007)	(0.20)	(0.8)	(0.6)
18°C					
Full ration	3	0.226 a	3.12	10.0	17.9
		(0.016)	(0.08)	(0.9)	(0.1)
Low ration	3	0.095 b	2.61	9.3	18.4
		(0.017)	(0.04)	(0.9)	(0.3)
Starved	3	−0.086 c	1.80	5.6	17.8
		(0.014)	(0.19)	(0.4)	(0.0)
21°C					
Full ration	3	0.246 a	2.88	10.4	21.1
		(0.017)	(0.16)	(1.0)	(0.9)
Low ration	3	0.071 b	2.45	8.6	21.1
		(0.045)	(0.13)	(0.1)	(0.7)
Starved	3	−0.118 c	1.86	6.7	21.2
		(0.063)	(0.07)	(0.5)	(0.7)

the average daily density of postlarvae in the neuston tows during each 2-wk sampling period, multiplied by 14 d. The total biweekly densities of postlarval lobsters were adjusted for the duration of the fourth instar at prevailing mean sea surface temperatures (based on MacKenzie 1988), using the formula of Scarratt (1964). Interannual differences in biweekly densities from the second half of June to the end of July during 1988–90 were tested with a one-way ANOVA.

In addition to the neuston tows, 5-min bongo tows (13-in. diameter, 333-μm mesh; paired nets, equipped with mechanical flowmeters) were made at a depth of 0–0.5 m at least twice a week, immediately after the neuston tows at each site in 1990. Zooplankton samples from the bongo nets were preserved in 5% formalin for at least a week and transferred to 70% isopropyl alcohol. In the laboratory, debris, juvenile fish, and gelatinous plankton which are not potential prey items (Juinio and Cobb 1992) were removed from the bongo samples. The most abundant zooplankton taxa in the samples were counted. Total wet weight was determined using a Sartorius top-loading balance after the samples were drained and blotted dry. Mean total zooplankton biomass (milligrams per cubic metre) at each site was estimated from the total wet weights in the paired bongo nets.

Field Growth Rates

The growth rates of field-caught postlarval lobsters in molt stages C and D_0 were estimated using the growth model developed from the laboratory data. Student t-tests were used to compare mean dry weights and growth rates between sites and months. Likewise, one-way ANOVAs (followed by Scheffe's F-tests, if significant differences were found) were computed to compare averages among sampling dates and years. Significant differences ($p < 0.05$) between frequency distributions of individual growth rates were determined by Kolmogorov–Smirnov two-sample tests (Sokal and Rohlf 1969). Pearson product moment correlations between the estimated biweekly total postlarval abundances and average growth rates were determined. Statistical analyses were conducted using SAS statistical programs (SAS Institute Inc. 1985) and Statview™ SE + Graphics (Feldman et al. 1987).

Results

Growth Model

The average growth rates and dry weights at molt stages C and D_0, RNA:DNA ratios, and temperature for the nine laboratory treatments are shown in Table 1. The mean batch growth rates attained at molt stages C and D_0 in the laboratory were not significantly different (t-test, $p = 0.675$); thus, the data for both molt stages were taken together to estimate the growth rates of wild postlarvae in the same molt stages using RNA:DNA ratios and temperature. Growth rates differed among the treatments (one-way ANOVA, $p = 0.0001$). At all temperature regimes, growth rates were highest in the full-ration treatment and lowest in the starved treatments (Scheffe's F-test, $p < 0.05$) (Table 1). The estimated average growth rates for each batch ranged from 0.14 to 0.26 mg protein·d^{-1} in the full-ration treatments from 0.03 to 0.12 mg protein·d^{-1} in the low-ration treatments, and from −0.04 to −0.16 mg protein·d^{-1} in the starved treatments. Postlarvae in the two fed treatments molted into stage V, while those in the starved treatment did not (Juinio et al. 1992). Fed postlarvae also were larger (8.6–11.0 mg dry

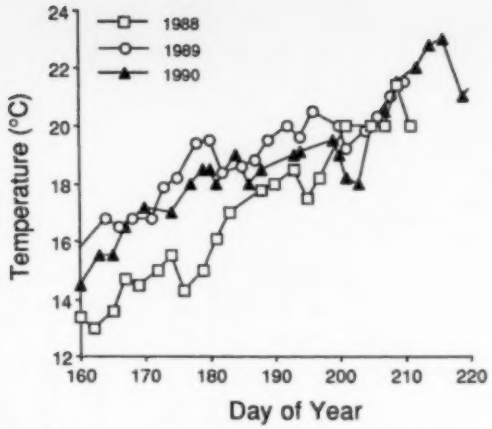

FIG. 2. Surface seawater temperatures over the sampling period 1988–90.

weight) than the starved postlarvae (5.6–6.7 mg dry weight) (Table 1).

Both temperature ($p = 0.003$ and RNA:DNA ratio ($p = 0.0001$) explained a significant amount of the variation in the growth rates (milligrams of protein per day) of postlarval lobsters at molt stages C and D_0. The relationship between average growth rates ($G_{C\text{-}D_0}$), RNA:DNA ratios (R), and temperature (T) for the three temperature regimes is described by

$$G_{C\text{-}D_0} = -0.747 + 0.218R + 0.014T \ (n = 21, r^2 = 0.818).$$

If we used only the data from 18 and 21°C treatments, temperature did not explain a significant amount ($p = 0.230$) of the variability in growth rates among treatments, while RNA:DNA ratios alone accounted for most of the variability in average growth rates among treatments ($p = 0.0001$, adjusted $r^2 = 0.859$). In this case, the relationship between average growth rates, RNA:DNA ratios, and temperature is described by

$$G_{C\text{-}D_0} = -0.778 + 0.268R + 0.01T \ (n = 18, r^2 = 0.864).$$

The growth rates of field-caught postlarval lobsters in molt stages C and D_0 were estimated using the second growth model based on tempertures 18–21°C which best represent the average (19.4 ± 1.3°C) and range (16.8–23.0°C) of sea surface temperatures when postlarvae were present in Block Island Sound during 1988–90 (Fig. 2). In addition, previous studies indicate that 15°C may be outside the range of metabolic compensation of postlarvae reared at a cyclic thermal regime of 17.5–22.5°C (Sastry 1980). In view of this, laboratory results in the 15°C treatment may be confounded by an acclimation period of unknown length (Juinio et al. 1992). Temperature was included in the model to take into account intra- and interannual variability in summer temperatures in the area. Surface seawater temperatures on the respective dates of collection were used to estimate individual growth rates.

Individual Variation

During each month at both sites, the dry weight of individual field-caught postlarvae in molt stages C and D_0 varied from 9.2 to 32.2 mg with an average of 18.2 ± 4.1 mg ($n = 385$) (Table 2). During most months, there was no correlation between dry weights and the estimated growth rates of individual postlarvae. However, there was a low but significant negative correlation between individual growth rates and dry weight for postlarvae sampled from Bell 4 in July 1988 and in June 1990 and July 1990 (Table 3).

At the Matunuck site during 1989 and in both sites during 1990, the frequency distributions of individual growth rates in June differed from those in July (Kolmogorov–Smirnov two-sample test, $p < 0.05$) (Fig. 3). The frequency distributions of estimated individual growth rates were different ($p < 0.05$) between the two sites only in July 1989 (Fig. 3c). When the data from both sites were pooled, the frequency distributions of June and July were different ($p < 0.05$) in both 1989 and 1990. Within each of these months, there

TABLE 2. Mean dry weight (mg ± sd) of postlarvae in molt stages C and D_0, collected from two nearshore sites in Block Island Sound for each month sampled during 1988–90 (n = number of individual samples). Means for both sites pooled also are shown.

	Bell 4	Matunuck	Pooled
1988			
July	17.6 ± 4.5 ($n = 51$)		
1989			
June	17.2 ± 2.9 ($n = 24$)	20.2 ± 4.7 ($n = 29$)	18.5 ± 4.1 ($n = 43$)
July	19.3 ± 2.6 ($n = 23$)	20.3 ± 3.3 ($n = 29$)	19.9 ± 3.0 ($n = 52$)
1990			
June	17.8 ± 4.9 ($n = 31$)	18.7 ± 4.5 ($n = 45$)	18.3 ± 4.7 ($n = 76$)
July	17.4 ± 3.9 ($n = 84$)	17.9 ± 3.5 ($n = 65$)	17.6 ± 3.7 ($n = 149$)
August	21.7 ± 10.8 ($n = 3$)	17.6 ± 2.7 ($n = 11$)	18.5 ± 5.2 ($n = 14$)

TABLE 3. Pearson's correlation coefficients (r) between the mean growth rates (mg protein·d^{-1}) and dry weight (mg) of individual postlarval lobsters at molt stages C and D_0 collected from two nearshore sites in Block Island Sound for each month sampled during 1988–90 (n = number of individual samples).* Significant correlations ($p < 0.05$).

	n	r	p
Bell 4			
1988			
July	51	−0.389	0.005*
1989			
June	24	0.211	0.322
July	23	0.088	0.688
1990			
June	31	−0.364	0.044*
July	84	−0.324	0.003*
Matunuck			
1989			
June	19	−0.230	0.343
July	29	0.114	0.555
1990			
June	45	−0.244	0.106
July	65	−0.206	0.100

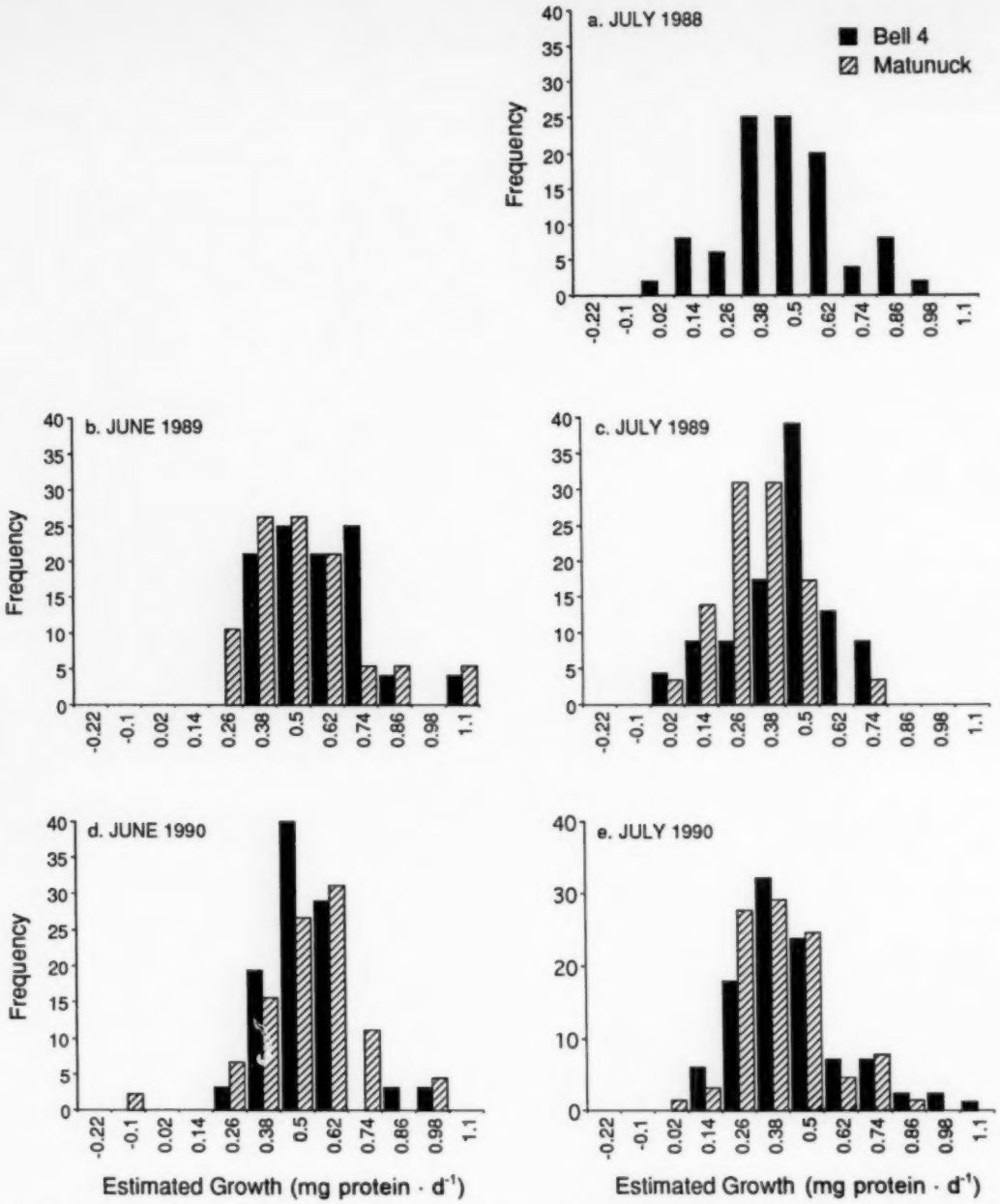

FIG. 3. Relative frequency distributions of estimated individual growth rates (0.12-mg intervals) of postlarval lobsters at molt stages C and D_0 from each site in Block Island Sound in months sampled during 1988–90.

was no significant difference between the frequency distributions in 1989 and 1990.

The lowest average growth rate among the replicate batches of postlarvae reared in the laboratory in the full-ration treatment was 0.22 mg protein·d^{-1}. Taking this as the lower limit for relatively well-nourished postlarvae, there were no poorly nourished animals sampled in June during 1989 and 1990 at either site (Fig. 3b and 3d) except for one individual in a starved condition (−0.15 mg protein·d^{-1}) collected from Matunuck in 1990. None of the postlarvae sampled in August 1990 (n = 11) had estimated growth rates indicative of poor nourishment. Almost all of the 22 poorly nourished postlarvae were sampled in July during the three years (Fig. 3a, 3c, and 3e). Poorly nourished postlarval lobsters composed less than 10% of the total postlarvae sampled each year (9.8% in 1988, 8.4% in 1989, and 3.8% in 1990).

TABLE 4. Mean estimated individual growth rates (mg protein·d^{-1} ± SE) of postlarvae in molt stages C and D_0, collected from two nearshore sites in Block Island Sound, off Rhode Island, for each month sampled during 1988–90 (n = number of individual samples). Means for both sites pooled and annual means are also shown.

	Bell 4	Matunuck	Pooled	Annual
1988				0.49 ± 0.20
July	0.49 ± 0.20 (n = 51)			
1989				0.47 ± 0.20
June	0.60 ± 0.18 (n = 24)	0.53 ± 0.20 (n = 19)	0.57 ± 0.19 (n = 43)	
July	0.45 ± 0.18 (n = 23)	0.34 ± 0.14 (n = 29)	0.39 ± 0.17 (n = 52)	
1990				0.47 ± 0.18
June	0.52 ± 0.14 (n = 31)	0.54 ± 0.20 (n = 45)	0.54 ± 0.18 (n = 76)	
July	0.45 ± 0.20 (n = 84)	0.41 ± 0.15 (n = 65)	0.43 ± 0.18 (n = 149)	
August	0.50 ± 0.27 (n = 3)	0.42 ± 0.07 (n = 11)	0.43 ± 0.13 (n = 14)	

Mean Growth Rates of Field-Caught Animals

The estimated mean growth rates of postlarvae caught in the field (Table 4) were all higher than the highest estimated mean growth rate, 0.26 mg protein·d^{-1}, at molt stage C and D_0 for postlarvae reared under the best conditions we could provide in the laboratory. Within each month, at both sites, mean growth rates were similar among sampling dates, with one exception, in June 1990 (ANOVA, $p = 0.007$). For this month, the estimated mean growth rate during the last sampling date (June 27) at Matunuck was lower (Scheffe's F-test, $p < 0.05$) than on the first sampling date (June 18). The mean growth rates between sites for each month were significantly different only in July 1989 (Table 4) when the mean growth rate at Bell 4 was significantly higher than that in Matunuck (t-test, $p = 0.0005$). When the data from both sites were pooled, the mean growth rate in June was higher than in July ($p < 0.0002$) in both 1989 and 1990. There was no difference in mean growth rates between June 1989 and June 1990. However, mean growth rates in July were different ($p = 0.018$) among years. The mean growth rate in July 1988 (0.49 mg protein·d^{-1}; Bell 4 only) was greater ($p < 0.05$) than that in July 1989 (0.39 mg protein·d^{-1}), but neither was significantly different from the mean growth rate in July 1990 (0.43 mg protein·d^{-1}).

Postlarval Densities, Zooplankton Biomass, and Growth Rates

The densities of lobster postlarvae, averaged over 2-wk periods, in June and July were significantly different among years (ANOVA, $p = 0.001$) (Fig. 4). The mean biweekly density in 1990 was significantly higher than that in 1988 and 1989 (Scheffe's F-test, $p < 0.05$). The patterns of abundance and the duration of the period when postlarvae were found in the neuston also varied among years, and peak density occurred at different times in each year. In 1988, the season was very short; postlarvae were not caught until the last week of June and postlarval densities were less than the other two years (Fig. 4). In all years the appearance of the postlarvae in the neuston tows occurred when sea surface temperature reached about 16°C. There was no correlation between the estimated mean biweekly growth rates and postlarval densities at either Bell 4 ($r = -0.33$, $p = 0.29$) or Matunuck ($r = 0.40$, $p = 0.40$) during the three years. Moreover, while there was a significant difference in annual total postlarval abundance among years, the estimated mean growth rates of postlarvae in molt stage C and D_0 for each year were not significantly different (Table 4).

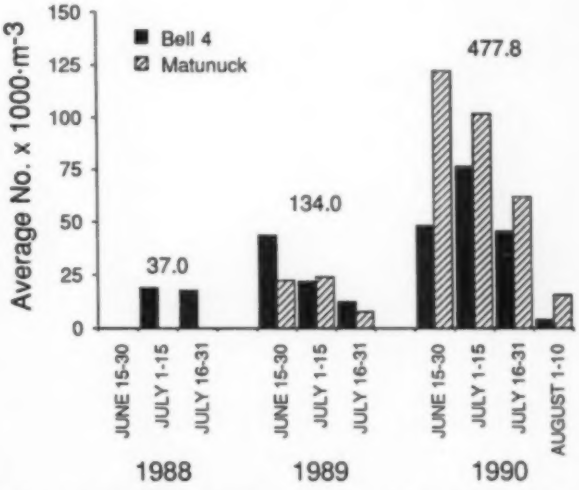

FIG. 4. Estimated biweekly average postlarval densities (adjusted for stage duration at prevailing sea surface temperatures) at each site during 1988–90. Numbers above the bars indicate the estimated annual average postlarval abundances for Bell 4 during 1988 and both sites during 1989 and 1990.

The predominant zooplankton taxa in the bongo samples during 1990 were copepods, decapod larvae, and fish eggs. Although the total zooplankton biomass estimates on some dates were highly variable between the two sites (range of

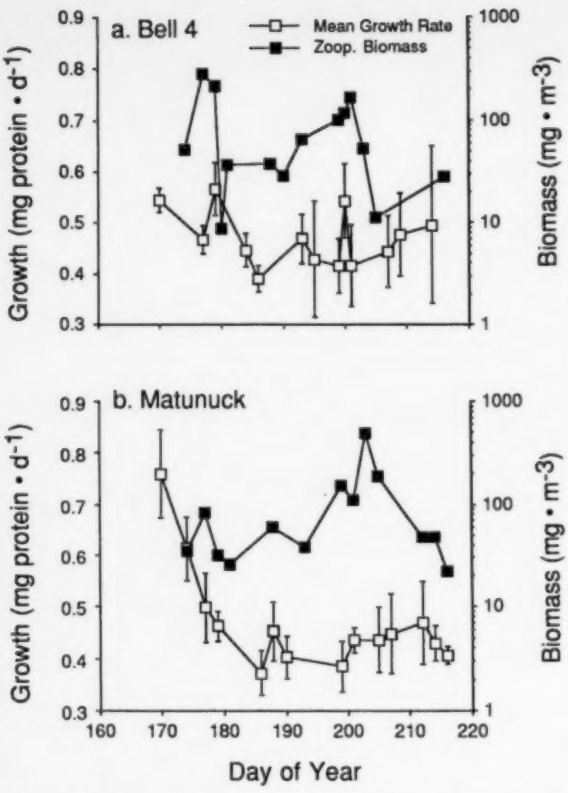

FIG. 5. Mean growth rates of postlarval lobsters at molt stages C and D_0 and estimated zooplankton biomass (wet weight) at Bell 4 and Matunuck, Block Island Sound, during 1990.

coefficients of variation 18.1–125.4%), periods of peak zooplankton abundance coincided at the two sites (Fig. 5). The first peak occurred June 22–27 and the second July 17–19, extending to July 23 in Matunuck. Neither a higher mean growth rate nor a lower incidence of poorly nourished postlarvae was found during, or shortly after, these periods of peak prey abundance at either site.

Discussion

A growth model based on RNA:DNA ratios and temperature was developed from laboratory data and used to estimate growth rates of individual wild *H. americanus* postlarvae. We found that 94% of all postlarvae in molt stages C and D_0 sampled in the field during the three years of the study had growth rates equal to or greater than the highest growth rates in the laboratory. The highest average estimated growth rate during a sampling month was 0.60 ± 0.18 mg protein·d^{-1}, more than twice the highest growth rate in the laboratory (0.26 ± 0.01 mg protein·d^{-1}), suggesting that postlarvae in the field grow better than laboratory-reared animals. This is consistent with the finding of Wilder (1953) that growth rates of larval lobsters in the laboratory, based on changes in total body length, were lower than those estimated from the field. It is apparent that the higher growth rates of field-caught postlarvae resulted in their larger sizes when compared with the laboratory-reared postlarvae. Hudon and Fradette (1988) also noted that larval and postlarval American and European lobsters in the wild were bigger than those reared in the laboratory. The average total dry weight of laboratory-reared postlarvae in the full-ration treatments in the three temperature regimes (11.17 ± 2.54 mg·individual^{-1}, $n = 113$, Juinio et al. 1992) was comparable with that found by Sasaki et al. (1986) for postlarval lobsters under similar feeding conditions. These are greater than the average dry weights of other laboratory-reared lobster postlarvae (e.g., 6.6 ± 2.3 mg·individual^{-1}, Logan and Epifanio 1978; 7.2 ± 0.3 mg·individual^{-1}, Capuzzo and Lancaster 1979). Moreover, survivorship in the experiments was very high (95% for all treatments) and all fed animals molted rapidly into stage V (Juinio et al. 1992). Taking this into account, we suggest that the discrepancy between the growth rates in the laboratory and the field is probably due to a higher quality of prey in the field. Despite the limitation of diet quality in the laboratory, the growth model based on the laboratory experiments can be used as a benchmark against which recent growth or nutritional status of individuals in the field can be compared. While maximum growth rates were not attained in the laboratory, growth rates indicative of starved and poorly nourished conditions in the laboratory can be used as a conservative basis to assess poor nutrition in the field.

The average growth rates of field-caught postlarvae were higher in June than in July 1989 and 1990; however, there were no apparent environmental conditions that would promote better growth earlier in the summer. The average temperatures during July (20.1 ± 3.0°C in 1989, 19.8 ± 1.0°C in 1990) were not much different from those in June (19.1 ± 0.9°C in 1989, 18.4 ± 0.6°C in 1990) and were well within the range for physiological compensation of postlarval lobsters (Sastry 1980). At 18–21°C, temperature did not explain a significant amount of the variability in the growth rates of laboratory-reared animals. Therefore, the lower average growth rates in July cannot be attributed to bioenergetic consequences of increasing summer temperatures. Furthermore, our laboratory studies under full-ration feeding conditions (Juinio 1991) and those of Carlberg and Van Olst (1976) suggest that growth rates would be expected to be higher with an increase in temperature, opposite to the trend we found in the field. Hudon and Fradette (1988) found maximum growth rates of larval *H. americanus* in the field to be highest during the warmest period of summer. Likewise, trends in the zooplankton biomass (an index of potential prey availability) during 1990 do not explain the higher growth rates in June that year. Zooplankton biomass was generally higher in July than in June, particularly at Matunuck. This indicates that factors other than food limitation and temperature contribute to the observed intraannual difference in mean growth rates at the nearshore areas.

Poorly nourished postlarvae (0.22 mg protein·d^{-1}) were absent in June. This suggests either that poorly nourished postlarvae experience high mortality rates or that all individuals were well nourished. Notably, the lower limits of individual growth rates in June (0.32 mg protein·d^{-1} in 1989, 0.20 mg protein·d^{-1} in 1990) were higher than would be expected for starved postlarvae, which showed negative growth in the laboratory, suggesting that starvation was not likely to be a major source of mortality. Poor nourishment affects the ability of crab larvae to avoid predation and to

capture prey (Cronin and Forward 1980); Paul and Paul 1980). If the same is true for postlarval lobsters, one possible source of the difference in the incidence of poorly nourished larvae between the two months is higher predation rates in June than in July. Predators of lobster larvae are thought to include all larger plankton-feeding fishes, ctenophores, and probably gulls and terns (Cobb and Wang 1985). However, no information is available on the relative abundance of these potential predators over the summer in the study area. Alternatively, field observations and studies on the swimming behavior of postlarval lobsters (Cobb et al. 1989a, 1989b; Rooney and Cobb 1991; Katz et al. 1994) do not eliminate the possibility that nearshore areas in Block Island Sound may receive subsidy from larvae spawned from other areas, including offshore waters. If this were the case, it is possible that the poorly nourished postlarvae collected later in the summer came from relatively "prey-poor" offshore waters. This hypothesis can be tested if nearshore stocks can be differentiated from other stocks using population genetic markers.

Despite the wide range of individual growth rates greater than 0.22 mg protein·d^{-1}, it is reasonable to assume that the total duration of the postlarval instar would be about the same among all individuals. In the laboratory, where growth rates were lower than in the field, we found no significant difference in the duration of the postlarval period in full-, and low-ration feeding treatments within each temperature despite significant differences in growth rates during the postmolt and intermolt periods (Juinio 1991; Juinio et al. 1992). Our experiments suggest that the wide variability of individual growth rates found in the field should not result in significant differences in the duration of the postlarval instar. This would be particularly true among postlarvae with growth rates greater than 0.22 mg protein·d^{-1}. Anger and Dawirs (1981) demonstrated that the molt cycle (i.e., development rate), unlike tissue growth, is independent of food availability after the "point of reserve saturation" is reached. This critical period in the development of decapod larvae generally occurred during the transition between molt stages C and D_0 (Anger 1987). Sasaki (1984) also found that if larval stages of *H. americanus* were starved after molt stage C, the duration of the instar did not increase. Consequently, the inference that rapid growth rate resulting from sufficient nourishment may decrease the vulnerability of early life stages to predation by reducing the duration in the plankton (Thorson 1950; Harding et al. 1983; Hudon and Fradette 1988) needs to be qualified for crustacean larvae. Other factors besides nutritional condition, such as finding suitable benthic habitat, are more likely to determine the duration in the plankton of the postlarval stage of *H. americanus*. Higher growth rates that result in larger size may affect vulnerability to predation, and this should be investigated.

The value of RNA:DNA ratios as an indicator of recent growth and thus in inferring short-term changes in prey availability in the field has been demonstrated for larval fishes. Buckley and Lough (1987) found a correlation between the abundance of prey and the nutritional condition of haddock (*Melanogrammus aeglefinus*) larvae at different sites on Georges Bank. A similar inference is reasonable in the case of postlarval lobsters because during the post-, and intermolt periods, RNA:DNA ratios reflect the feeding success of an organism during the past few (2–6) days (Juinio et al. 1992). Postlarval lobsters were found at all molt stages, including the earliest stages, in our study area, indicating that at least a proportion of the postlarvae we collected had been growing in the area. It is reasonable to assume that many of the postlarvae used in this study were feeding in the nearshore area for a few days. Thus, the absence of an increase in mean growth rate of postlarvae during, or shortly after, periods of high potential prey availability suggests that postlarval lobsters caught at these nearshore sites were not food limited. This is consistent with the generally very high growth rates of postlarvae in the field.

Poorly nourished postlarvae composed less than 6% of the 385 wild postlarvae in this study. However, their presence, including one postlarva in a starved condition, indicates that a small proportion of the population was food limited, resulting in lower growth rates. While mortality due to starvation cannot be ruled out, it is unlikely to be a major source of mortality of postlarvae in the field. In the laboratory, postlarvae survived for at least 2 wk (or until termination of the experiment, up to 29 d in one case) even when starvation was initiated immediately after ecdysis (Juinio et al. 1992).

RNA:DNA ratios, together with temperature, provide a useful tool to determine recent growth during the post- and intermolt period, and therefore, the relative nutritional condition of individual postlarval lobsters in the field. The general absence of a significant correlation between individual total dry weight and growth rate indicates that there was no bias in the estimated growth rates due to size. This is not surprising, since growth rates based on RNA:DNA ratios are not affected by variation in cell numbers (Bulow 1987). This would be particularly true within the same life stage. This is important because field-caught postlarvae varied greatly in size. Because biochemical and physiological changes over the molt cycle vary among developmental stages of *H. americanus* (Sastry 1980; Sasaki et al. 1986), the usefulness of RNA:DNA ratios should be evaluated for other instars before application to field-caught animals.

In crustaceans, where the duration of the instar is independent of food availability beyond the "point of reserve saturation" (Anger and Dawirs 1981), the common assumption that growth rates are directly related to survival via instar duration (Thorson 1950; Harding et al. 1983; Hudon and Fradette 1988) needs to be reconsidered. Because a higher growth rate may result in a larger size but not necessarily a decrease in the length of time spent in the plankton, the relationships among growth rate, size, behavior, and survival need to be elucidated to understand how nutrition affects postlarval recruitment.

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Transport of *Bacillus thuringiensis* var. *israelensis* and Its Effect on Drift and Benthic Densities of Nontarget Macroinvertebrates in the Susquehanna River, Northern Pennsylvania

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The microbial pesticide *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) was applied aerially to suppress larval black fly populations in the Susquehanna River, Pennsylvania. We quantified (1) the passage and retention of *B.t.i.* spores through a 1.5-km reach and (2) changes in black fly and nontarget macroinvertebrate abundances in the drift and benthos. *B.t.i.* exposure in the treatment riffle was 1.16×10^5 min-spores/mL or 16 min-mg/L in 1989 and 5.20×10^4 min-spores/mL or 4.4 min-mg/L in 1990. Retention of *B.t.i.* within the reach was low. While black fly drift from the treatment riffle increased markedly, *B.t.i.* appeared to have little effect on drift densities of most nontarget macroinvertebrates during the 48-h period following application. Similarly, benthic densities of black flies in the treatment riffle decreased significantly 1 d after the *B.t.i.* application whereas benthic densities of most nontarget taxa in the treatment riffle and depositional zone appeared unaffected 1, 7, and 28 d following *B.t.i.* application. Responses potentially related to *B.t.i.* exposure were observed for the aquatic lepidopteran *Petrophila* and the chironomid *Polypedilum*. These results suggest that the application of *B.t.i.* on the Susquehanna River markedly reduces black fly populations, but does not negatively affect most nontarget macroinvertebrates.

Le pesticide microbien *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) a été appliqué par la voie des airs pour éliminer les populations larvaires de mouches noires dans la rivière Susquehanna, en Pennsylvanie. Nous avons quantifié 1) le passage et la rétention des spores de *B.t.i.* sur un tronçon de 1,5 km et 2) les changements dans l'abondance des mouches noires et des macroinvertébrés non visés dans le rhéoplancton et le benthos. Le traitement au *B.t.i.* dans le radier était de $1{,}16 \times 10^5$ min-spores/mL ou 16 min-mg/L en 1989 et $5{,}20 \times 10^4$ min-spores/mL ou 4,4 min-mg/L en 1990. La rétention de *B.t.i.* dans le tronçon de rivière était faible. Tandis que la dérive de mouches noires à partir du radier traité a augmenté nettement, le *B.t.i.* semble avoir eu peu d'effet sur les densités de la dérive de la plupart des macroinvertébrés non visés pendant la période de 48 h qui a suivi l'application. De même, les densités benthiques de mouches noires dans le radier traité ont baissé nettement 1 jour après l'application de *B.t.i.*, tandis que les densités benthiques de la plupart des taxons non visés dans le radier traité et la zone d'alluvions ne semblaient pas affectées 1, 7 et 28 jours après l'application de *B.t.i.* On a observé des réactions qui peuvent être liées à l'exposition au *B.t.i.* chez le lépidoptère aquatique *Petrophila* et le chironomidé *Polypedilum*. Ces résultats semblent indiquer que l'application de *B.t.i.* dans la Susquehanna réduit de façon marquée les populations de mouches noires, mais n'a pas d'effet négatif sur la plupart des macroinvertébrés non visés.

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Bacillus thuringiensis var. israelensis de Barjac, Serotype H14 (*B.t.i.*), is an aerobic, spore-forming bacterium that produces a crystalline protoxin. The protoxin becomes activated in the digestive tract of some aquatic insects, causing rapid disruption of cellular function, and eventually paralysis and death (Gaugler and Finney 1982; Singh et al. 1986; Cheung et al. 1987; Chilcott et al. 1990). Larval black flies have proven to be especially susceptible to *B.t.i.* This has resulted in *B.t.i.* being used extensively and effectively to regulate population sizes of larval black flies in North American streams and rivers (Molloy 1990), thereby reducing the public annoyance and economic loss associated with the biting activities of adult black flies.

If *B.t.i.* is toxic to other macroinvertebrates (i.e., nontarget species) that ingest it, application of *B.t.i.* could potentially affect a large proportion of the macroinvertebrate assemblage in a stream or river because it is readily available to most species. For example, *B.t.i.* could be ingested by filter-feeding species collecting *B.t.i.* and other particles suspended in the current, by grazers, collector-gatherers, and/or shredders feeding on algae or detritus that may include *B.t.i.* that settles out of the water column, or by predators or detritivores consuming animals containing *B.t.i.* Besides toxic effects on nontarget macroinvertebrates, the removal of black flies by the application of *B.t.i.* represents a disturbance to biotic interactions and trophic structure associated with black flies.

Previous studies found that *B.t.i.* had little or no effect on most nontarget macroinvertebrates examined (Merritt et al. 1989; Molloy 1990). However, with few exceptions (the Marahoué River, Ivory Coast (Lacey et al. 1982) and the Orange River, South Africa (de Moor and Car 1986)), these

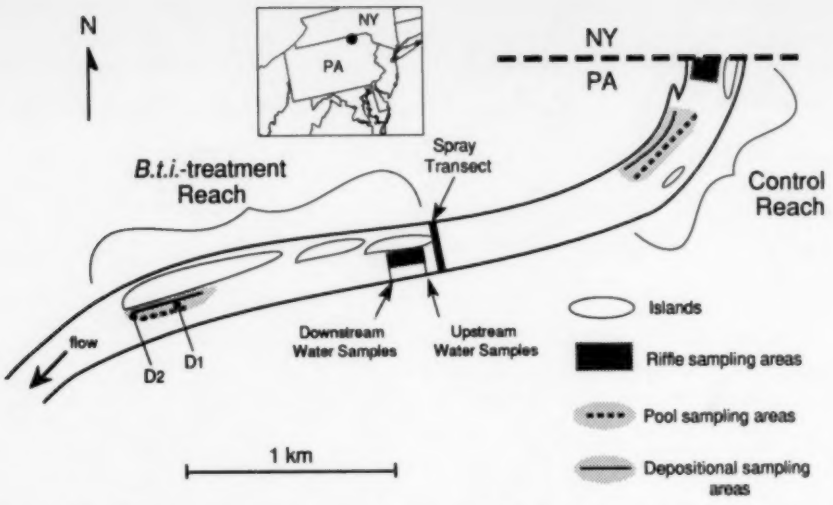

FIG. 1. Map showing control and treatment reaches on the Susquehanna River. Inset shows location in Pennsylvania, U.S.A. D1 and D2 are sites where additional water samples were taken in 1990.

studies were conducted in streams and a few small rivers. This focus on smaller lotic ecosystems parallels research in lotic ecology overall: Hynes (1989) observed that less than 5% of all papers published on ecological topics associated with running waters address large rivers. Hynes (1989) also suggested caution when extrapolating results from streams to large rivers, presumably because of the limited information available on large rivers and the potential differences between streams and large rivers. Two factors that differ greatly are hydrologic conditions and the composition of macroinvertebrate faunas, both of which are important in addressing the effects of *B.t.i.* on lotic ecosystems. Greater depth and velocity in large rivers should increase transport distances of small particles such as *B.t.i.* (Cushing et al. 1993), which would result in significant *B.t.i.* exposures much farther downstream of the application point. Few direct measures of *B.t.i.* transport have been made in streams or large rivers, and deposition patterns remain unexamined. Moreover, the effect of *B.t.i.* on nontarget macroinvertebrates found in large rivers is still relatively unknown because many macroinvertebrate species (and even genera) characteristic of large rivers often do not inhabit smaller streams (Hawkes 1975). Finally, as pointed out by others (e.g., Gaugler and Finney 1982; Merritt et al. 1989; Molloy 1990), results in many previous studies of *B.t.i.* can be difficult to interpret because of improper experimental design (sample replication and controls were inadequate or absent) and limited taxonomic resolution (e.g., Chironomidae were identified to family or subfamily).

Our study examined the effects of large-scale applications of *B.t.i.* in the Susquehanna River, an important cultural and natural resource in Pennsylvania that sustains many recreational, fisheries, and wildlife uses. With the exception of the Marahoué River (Lacey et al. 1982), discharge in the Susquehanna River is greater (often by a factor of 100–1000) than in other *B.t.i.* studies (Molloy 1990). *B.t.i.* is applied to all major riffle areas in the main stem of the Susquehanna River in Pennsylvania approximately every 10 d between May and September to control black flies. Our objectives were to (1) document the movement and retention of *B.t.i.* spores following the aerial application of *B.t.i.* to the Susquehanna River and (2) examine the effects of that *B.t.i.* application on both black fly and nontarget macroinvertebrate drift and benthic densities in the Susquehanna River. These results were interpreted with regard to changes in the structure and function of the river ecosystem.

Study Site

The study site was 3.2-km section on the North Branch of the Susquehanna River (41°59′54″N, 76°28′30″W, elevation = 228.6 m a.s.l.), just upstream of Sayre, Bradford County, Penn. (Fig. 1). Mean discharge at this eighth-order site was 214.1 m^3/s during the 1989–90 water years (range = 19.3–1350.9 m^3/s; USGS Gaging Station 01515000). Water temperature was 21.5–26.5°C during the study periods. The stream bed had a low gradient (<0.1%) that resulted in short (200–400 m long) riffles separated by expansive pools and depositional zones (often >1 km long). Two riffle/pool-depositional zone sequences were in the study site: the upstream sequence was designated the control reach and the downstream sequence was designated the treatment reach (Fig. 1). River width was ≈150 m at the control riffle and 88–100 m at the treatment riffle. Characteristics of riffles, pools, and depositional zones sampled during summer baseflow conditions were as follows: (1) a riffle was a shallow (0.2–0.7 m), fast-flowing (0.4–0.7 m/s) area that had cobble substrate (longest axis = 64–256 mm); (2) a pool was a deep (0.5 to >3.0 m), slow-flowing (<0.1 m/s) area that had cobble substrate and was near the center of the main river channel; (3) a depositional zone was a shallow (0.3–1.5 m), slow-flowing (<0.1 m/s) area that had silt/cobble substrate and was located within 5 m of the river bank.

Within the control reach, benthic samples were collected from a 20 × 80 m area of riffle, a 5 × 430 m area in the depositional zone downstream of the riffle, and in the pool

that paralleled the depositional zone (Fig. 1). Similarly, benthic samples from the treatment reach were collected from a 10 × 160 m area of riffle, a 5 × 350 m area in the depositional zone downstream of the riffle, and the pool adjacent to the downstream portion of the depositional zone (Fig. 1).

To our knowledge, *B.t.i.* had never been applied to the control reach. *B.t.i.* had been applied to the treatment reach in previous years, but was not applied in 1989 or 1990 prior to the experimental application.

Materials and Methods

Experimental Application of *B.t.i.*

On 7 August 1989, 56.8 L of an aqueous *B.t.i.* suspension (1200 International Toxic Units (ITU)/mg or 1.28×10^9 ITU/L, VectoBac AS/12AS, Abbott Laboratories, North Chicago, Ill.) was mixed with a rhodamine WT dye solution (1.5 L of 20% rhodamine WT dye, diluted with 20 L of distilled water) and sprayed from a helicopter into the Susquehanna River ≈100 m upstream of the benthic sampling area of the treatment riffle. Rhodamine was introduced simultaneously with the application of *B.t.i.* spores as a conservative tracer so that retention of *B.t.i.* spores could be calculated. River discharge at the time of the 1989 application was 76.7 m^3/s. On 17 August 1990, 45.4 L of *B.t.i.* suspension (1200 ITU/mg) was mixed with 1.0 L of 20% rhodamine WT dye and sprayed by helicopter into the Susquehanna River ≈100 m upstream of the treatment riffle. River discharge at the time of the 1990 application was 60.9 m^3/s. We were unable to obtain reliable estimates of *B.t.i.* spore concentrations in undiluted VectoBac.

Collection of Water Column and Benthic Samples for *B.t.i.* Analysis

Water column samples (250 mL) for rhodamine analysis and quantification of *B.t.i.* spores were collected along a transect located at the upstream limit of the treatment riffle and along a transect located 190 m (1990) or 200 m (1989) downstream of the upstream transect (Fig. 1). Three positions along each transect were monitored in 1989, while four positions were monitored in 1990. Additional water samples were collected in 1990 at locations 1.25 and 1.5 km (sites labelled D1 and D2, respectively) downstream of the upstream transect (Fig. 1). In 1989, a subsample of each water sample was transferred to a sterile test tube, heated to kill vegetative cells, and transported on ice to the laboratory for enumeration of viable spores. The remaining water was preserved with 5 mL of 37% formalin. In 1990, entire samples were preserved with formalin in the field.

Benthic samples were collected from riffles, pools, and depositional zones in 1989 and from riffles and depositional zones in 1990. Ten samples from treatment areas and five samples from control areas were collected before and after *B.t.i.* application. Each riffle and pool sample contained epilithic material scraped from three cobbles (total area = 85.5 cm^2). Each depositional zone sample contained surface silt (the top 2–4 mm) from three core samples (total area = 5.4 cm^2) collected using a syringe barrel with the tip removed. Gravel underlying cobbles was also sampled at five riffle stations in 1990 by removing surface cobbles from a portion of streambed and working a Hess sampler (0.088 m^2) lined with a metal sleeve into the gravel to isolate the area. The gravel was stirred vigorously to suspend fine particulates, and 1 L of water was collected. Water depth in the sampler was measured for the calculation of water volume in the sampler. All benthic samples were preserved with formalin.

Quantification of *B.t.i.* Spores

B.t.i. spores were enumerated by epifluorescence microscopic counts of formalin-fixed cells visualized with fluorescent antibodies (FA) (Bohlool and Brock 1974). *B.t.i.* antiserum was produced commercially (Cocalico Biological, Reamstown, Penn.) from killed spores and vegetative cells. Attempts to prepare antiserum against *B.t.i.* proteinaceous crystals (the actual toxin) were unsuccessful because titers in agglutination tests were never sufficiently high for use with field collections. Antiserum was fractionated and purified, using agarose gel chromatography to obtain an IgG fraction (Bio-Rad Laboratories; Goldman 1968). Conjugation with 5-(4,6-dichlorotriazin-2YL)-aminofluorescein hydrochloride (DTAF; Sigma Chemical Co.; Lewis et al. 1964) produced a FA that was tested for specificity with closely related *Bacillus cereus* (ATCC 21281, 6464, and 14579). Cross-absorption with whole-cell preparations was used to remove cross-reactivity (Apel et al. 1976).

Water column samples were sonicated and filtered onto a polycarbonate membrane (Hobbie et al. 1977). Benthic samples of cobble, silt, and gravel were concentrated by centrifugation, resuspended, sonicated, and the large debris allowed to settle through 30% glycerol. Filters from the water column were coated with gelatin and those from benthic samples were coated with rhodamine-conjugated gelatin to control nonspecific staining (Bohlool and Schmidt 1968) during the FA staining procedure (Ward and Perry 1980). Blanks were performed to test for contamination of stains, reagents, and the filtering apparatus. FA-positive spores were enumerated in 20 or 40 fields with an epifluorescence microscope. Enumeration of viable spores from the water column with the heat shock and spread plate technique (Gibbs et al. 1986) was performed in 1989 to confirm the FA method.

Exposure and Retention of *B.t.i.*

Rhodamine concentrations in water column samples preserved with formalin were measured using a Turner model 10-005R fluorometer for retention calculations. Observed rhodamine and *B.t.i.* spore exposures were calculated by integrating the area under the curve relating rhodamine or *B.t.i.* concentration as a function of time for each sampling position. To evaluate retention of *B.t.i.* in riffles, we computed the ratio of *B.t.i.* exposure to rhodamine exposure for each sampling position and compared the ratios from downstream positions with those from corresponding upstream positions. Rhodamine was assumed to behave as a conservative tracer of water movement and was used to compensate for lateral mixing within a riffle.

Collection of Drifting and Benthic Macroinvertebrates

Macroinvertebrate drift was sampled at selected times during a period spanning 1 d before and 2 d after the *B.t.i.* application using 350-μm-mesh nets that were 24 cm wide,

75 cm tall, and 1 m long. Merritt et al. (1989) found that a smaller size mesh did not significantly affect their analyses of drift response to *B.t.i.* Drift was measured along two lateral transects, one 10 m downstream of the benthic sampling area in the control riffle and one 20 m downstream of the benthic sampling area in the treatment riffle. Four drift nets were spaced 2–4 m apart along each transect. Water depth and current velocity immediately in front of each net were measured at the beginning of a sample period. Nets were removed from the river after 15 min and the captured material was preserved with 95% ethanol. Sampling at the control and treatment riffles was not synchronous in 1989 because of equipment difficulties, but was synchronous in 1990. Drift sample periods in 1989 began approximately 24, 18, 12, and 6 h before the *B.t.i.* application (i.e., at 08:23 and 10:03, 14:03 and 15:33, 21:10 and 19:40, and 02:05 and 03:55 Eastern Standard Time in control and treatment riffles, respectively) and 0.5, 1, 2, 6, 12, 18, 24, and 48 h after the *B.t.i.* application (i.e., at 09:15 and 08:45, 10:01 and 09:37, 10:42 and 10:41, 14:23 and 14:09, 19:59 and 18:45, 03:55 and 01:55, 09:13 and 09:52, and 07:59 and 08:39, respectively). Drift sample periods in 1990 began approximately 26, 21, 15, and 9 h before the *B.t.i.* application (i.e., at 08:30, 13:30, 19:30, and 01:30) and 0.5, 1, 2, 3, 9, 15, 22, and 46 h after the *B.t.i.* application (i.e., 10:52, 11:23, 12:23, 13:20, 19:30, 01:30, 08:30, and 08:30, respectively). Sampling times preceding the *B.t.i.* application are labelled as negative times.

Benthic macroinvertebrates were sampled in riffles (1989) and in depositional zones (1990) 2 d before and 1, 7, and 28 d after the *B.t.i.* application. Eight riffle samples were collected in a stratified random pattern with a Hess sampler (0.088 m^2, 500-μm mesh) on all dates, except when rain elevated the river stage 1 d after the *B.t.i.* application and a dome sampler (0.16 m^2, 500-μm mesh) was required. The dome sampler was also used to collect eight depositional zone samples on all dates along a transect that ran the length of each depositional zone. All benthic macroinvertebrate samples were elutriated in the field through a 250-μm-mesh sieve and preserved with 5% buffered formalin.

Each macroinvertebrate sample was split into 10 subsamples because macroinvertebrates were so numerous (some had >3000–5000 individuals/benthic sample, >1000 individuals/drift sample). We examined one drift subsample in 1989, two drift subsamples in 1990, and two benthic subsamples in both 1989 and 1990.

Statistical Analysis of Macroinvertebrate Data

The statistical distributions of macroinvertebrate data usually differed significantly from the normal (Kolmogorov–Smirnov test), as is characteristic with populations having a clumped spatial distribution (Elliott 1977). The $\ln(X+10)$ transformation (where X=number per 100 m^3 for drift or number per square metre for benthic density, converted from number per sample) was applied to all data prior to analysis.

A two-way (reach by time) analysis of variance (ANOVA) was used to examine macroinvertebrate drift data. An effect of the *B.t.i.* application was observable only in the treatment reach on postapplication sampling times. Therefore, the null hypothesis of no treatment effect was rejected if there was a significant interaction between spatial and temporal effects. If a significant interaction occurred, the effects at individual sampling times were assessed by linear contrasts of the interaction terms (SAS Institute, Inc. 1985). Because drift from the control riffle exhibited a pronounced diel periodicity typical of macroinvertebrates, postapplication data were compared with preapplication data taken near the same time of day. For example, in 1990, 3-h postapplication data were compared with data collected 21 h prior to the *B.t.i.* application (i.e., both sample periods began at 14:30). Fog over the river on the morning of 17 August 1990 delayed the application of *B.t.i.* for almost 2 h, from 09:30 to 11:22. No comparable data for this sample time were collected prior to the *B.t.i.* application. As a result, the 0.5-, 1-, 2-, 22-, and 46-h data were compared with mean differences between the control and treatment riffles during the −26- and −21-h periods.

In our interpretation of significant contrasts for nontarget species, we assumed that a toxic effect of *B.t.i.* on nontarget species would result in a drift response similar to the increased drift observed for black flies (which are known to be susceptible to *B.t.i.*). This would presumably be accompanied by a significant decrease in benthic densities in the treatment reach relative to the control reach. Changes in drift that were not similar to the response of black flies were interpreted as naturally occurring spatial/temporal variation in the diel drift pattern. This conservative approach reduces the risk of type I errors that could result from natural variation and the examination of multiple taxa.

A similar analysis was used to test for effects of the *B.t.i.* application on benthic densities. If a significant interaction appeared, the effects on individual sampling dates were assessed by linear contrasts of the interaction terms for pre- and postapplication dates. For example, the hypothesis of no treatment effect on Day 1 was tested by contrasting Day 1 with Day −2. We assumed that a toxic effect of *B.t.i.* on nontarget species would result in a significant decrease in benthic densities in the treatment reach relative to the control reach. This would presumably be accompanied by a significant increase in drift from the treatment reach relative to the control reach, similar to that commonly observed for black flies. Higher benthic densities in the treatment reach relative to the control reach on postapplication dates were interpreted as evidence of naturally occurring spatial/temporal variation in benthic densities and recruitment.

Results and Discussion

Specificity of FA

Antibodies developed for *B.t.i.* spores had only limited cross-reactions with spores of *B. cereus* (ATCC 6464). We felt this was not a problem because only a few spores were stained in a preparation containing hundreds of *B. cereus* spores per microscopic field. Further evidence that cross-reactions were not common is the fact that no fluorescing cells were found in benthic material from the treatment reach before spraying and from the control reach after spraying in 1989. Viable spore counts from water samples confirmed FA observations, although viable spore counts were slightly lower (Fig. 2). Lower viable counts suggest that not all spores were viable and/or some spores clumped together (sonication was used to reduce clumping in FA preparations whereas sonication was not used in viable preparations).

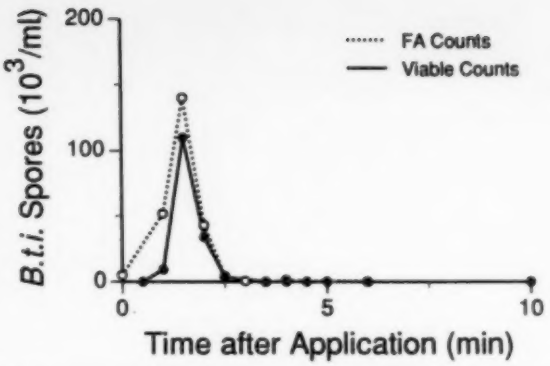

FIG. 2. Comparison of *B.t.i.* spore concentrations estimated from counts of FA-stained (dotted line) and viable (solid line) spores in water samples from the upstream site on the south longitudinal sampling transect during the first 10 min following the 1989 *B.t.i.* application. Coefficient of variation averaged 110% (n = 14), 60% (n = 11), 30% (n = 8), and 17% (n = 3) for samples with mean FA spore counts of <2500, 2500 to <10 000, 10 000 to <60 000, and >60 000 spores/mL, respectively. When viable spore counts were >1000 spores/mL (n = 26), viable spore counts were 64.0 ± 29.4% of FA counts.

B.t.i. Concentration in the Water Column, Exposure, and Retention

We assume that movements of *B.t.i.* spores parallel movements of the proteinaceous crystals (the actual toxin) and can be used to describe transport, exposure, and retention patterns of crystals following *B.t.i.* applications.

Treatment riffle in 1989

B.t.i. entered the treatment riffle 1–2 min after application, with peak spore concentrations at the top of the treatment riffle of 9.32×10^4 to 2.33×10^5 spores/mL approximately 0.5 min later (Fig. 3). *B.t.i.* reached the bottom of the riffle 5–6 min after application, with peak concentrations of 3.48×10^4 to 8.49×10^4 spores/mL. Spore concentrations in the treatment riffle had returned to pretreatment levels after approximately 9 min.

Based on the average rhodamine exposure at the six sampling positions (92 min-µg/L, i.e., equivalent to 92 µg/L for 1 min; Table 1) and the rhodamine to *B.t.i.* ratio in the spray solution (0.17 mg *B.t.i.*/µg rhodamine), average *B.t.i.* exposure observed across the treatment riffle was 16 min-mg/L or 19 min-ITU/mL. This empirically derived value was similar to the theoretically predicted exposure of 13 min-mg/L or 16 min-ITU/mL resulting from the application of 56.8 L of *B.t.i.* suspension at a river flow of 76.7 m^3/s. When expressed as *B.t.i.* spore counts, average *B.t.i.* exposure observed in 1989 was 1.16×10^5 min-spores/mL (Table 1). Black flies and nontarget macroinvertebrates in the benthic sampling area (i.e., near the north longitudinal transect) had an average *B.t.i.* exposure of 16 min-mg/L or 1.54×10^5 min-spores/mL (Table 1). The ratios of spore exposure to rhodamine exposure at the downstream positions ranged from 92 to 228% of those at corresponding upstream positions (Table 1), with no consistent difference between upstream and downstream (P = 0.43, two-way ANOVA). Thus, the quantity of *B.t.i.* spores retained could not be precisely estimated from these data. While some retention of *B.t.i.* spores occurred in the riffle (see below), it appears to represent only a small proportion of the spores applied.

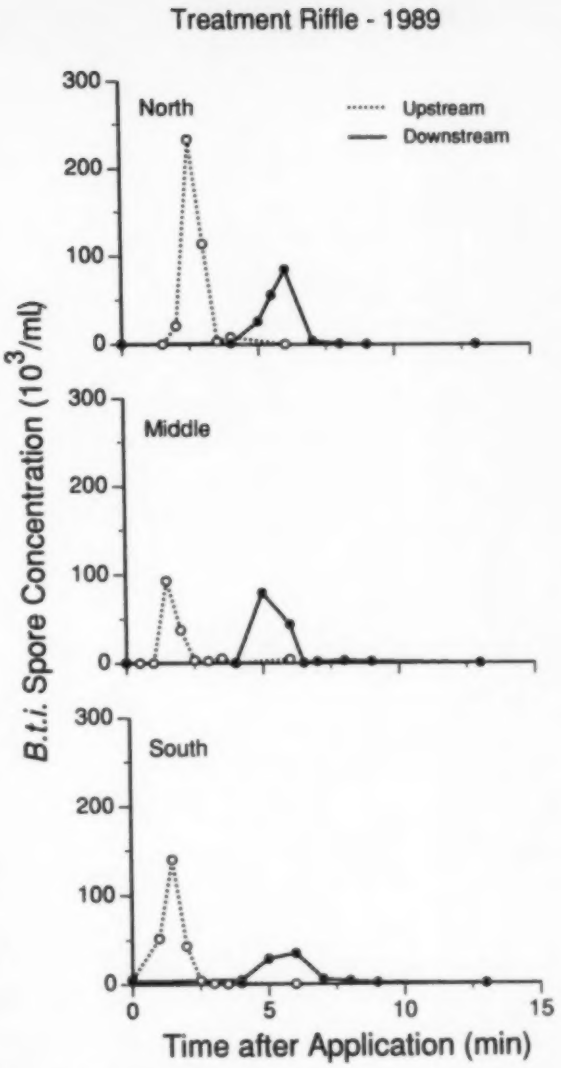

FIG. 3. Concentration of *B.t.i.* spores in water samples from upstream and downstream sites on each longitudinal sampling transect (i.e., north, middle, south) in the treatment riffle during the 15 min following the 1989 *B.t.i.* application.

Treatment riffle in 1990

Patterns of *B.t.i.* spore transport through the treatment riffle in 1990 were similar to those observed in 1989, although peak spore concentrations occurred later and were only one third to one half the peak concentrations in 1989 (Fig. 3 and 4). *B.t.i.* first reached the treatment riffle 2–2.75 min after application, with peak concentrations at the top of the treatment riffle of 3.45×10^4 to 7.12×10^4 spores/mL approximately 0.5–1.75 min later (Fig. 4). *B.t.i.* reached the bottom of the treatment riffle 5–6.5 min after application, with peak concentrations of 1.72×10^4 to 4.42×10^4 spores/mL.

TABLE 1. Rhodamine and *B.t.i.* spore exposures for longitudinal transects in the treatment riffle.

	1989				1990				
	North	Middle	South	$\bar{X}$ for riffle	North	Mid-north	Mid-south	South	$\bar{X}$ for riffle
Rhodamine (min-μg/L)									
Upstream	122	89	119		36	22	15	13	
				92					22
Downstream	72	66	84		38	21	18	11	
$\bar{X}$ for transect	97	78	102		37	22	16	12	
Spores (min-spores/mL)									
Upstream	1.90×10^5	0.70×10^5	1.20×10^5		6.91×10^4	5.62×10^4	5.28×10^4	4.34×10^4	
				1.16×10^5					5.20×10^4
Downstream	1.17×10^5	1.19×10^5	0.78×10^5		8.64×10^4	3.40×10^4	3.21×10^4	4.23×10^4	
$\bar{X}$ for transect	1.54×10^5	0.95×10^5	0.99×10^5		7.78×10^4	4.51×10^4	4.25×10^4	4.29×10^4	
Spores:rhodamine (spores/pg)									
Upstream	1.56	0.79	1.01		1.94	2.51	3.45	3.39	
				1.29					3.48
Downstream	1.63	1.80	0.93		2.25	1.61	1.80	3.93	
Ratio of downstream to upstream (%)	104	228	92		116	64	52	116	

Spore concentrations had returned to pretreatment levels after 11 min.

Observed exposure determined from an average rhodamine exposure of 21.8 min-μg/L (Table 1) and a spray mixture of 0.20 mg *B.t.i.*/μg rhodamine was 4.4 min-mg/L. This was much lower than the predicted exposure of 13 min-mg/L calculated from 45.4 L of *B.t.i.* sprayed at a river flow of 60.9 m^3/s, which would have been similar to the 1989 exposure. The discrepancy between observed and predicted exposures may have been caused by inadequate mixing of rhodamine and *B.t.i.* in the spray tank and/or a failure to expel all of the material from the spray tank. Unlike 1989, only 20–40% of the rhodamine put into the spray tank was accounted for in the river. Exposures based on counts of individual *B.t.i.* spores in water samples (Table 1) average 5.20×10^4 min-spores/mL over the eight sampling positions, or about half the exposure in 1989. Average *B.t.i.* exposure for black flies and nontarget organisms in the benthic sampling area (i.e., near the mid-north longitudinal transect) was 4.51×10^4 min-spores/mL.

The ratios of spore exposure to rhodamine exposure at the downstream positions ranged from 52 to 116% of those at the upstream stations (Table 1), with no consistent difference between upstream and downstream (P=0.47, two-way ANOVA). As in 1989, it appears that retention of spores in the riffle was limited.

Treatment depositional zone in 1990

B.t.i. did not reach D1 until 50 min after the application, and it took approximately 50 min for spore concentrations there to return to near background (Fig. 5). Spore exposure at D1 was 1.33×10^5 min-spores/mL, or 54% greater than the maximum observed in the riffle (8.64×10^4 min-spores/mL at the downstream north position). Rhodamine exposure at D1 (42 min-μg/L) was 9% greater than the greatest exposure observed in the riffle (Table 1).

B.t.i. concentrations began to increase at D2 at about the same time that *B.t.i.* returned to near background concentrations at D1, peaking 110 min after application (Fig. 5). Sampling was unfortunately discontinued before concentrations returned to background levels because rhodamine concentrations at D2 were too low for visual detection. This prevented estimation of exposure at D2. However, the spore to rhodamine ratio for the period sampled was 2.04 spores/pg, which was comparable with the highest levels observed in the riffle and approximately 66% of that at D1 (3.10 spores/pg).

Occurrence of *B.t.i.* in Benthic Samples

Following *B.t.i.* application in 1989, *B.t.i.* spores were not detected by FA on cobble surfaces in the treatment riffle or on the sediment surface in the depositional zone. However, some spores settled in the treatment pool (mean = 4.31×10^3 spores/cm^2; range = 0 to 1.30×10^4 spores/cm^2). Because *B.t.i.* was not found in the treatment riffle and depositional zone after the spray, only samples taken before spraying from the treatment pool were examined, and none contained *B.t.i.* Similarly, no spores were found in selected samples collected from the control riffle, pool, and depositional zone after *B.t.i.* application to the treatment reach.

B.t.i. spores were also not detected in epilithic samples collected from the treatment riffle following *B.t.i.* application in 1990. However, spores were found in all gravel samples taken from under cobbles in the treatment riffle

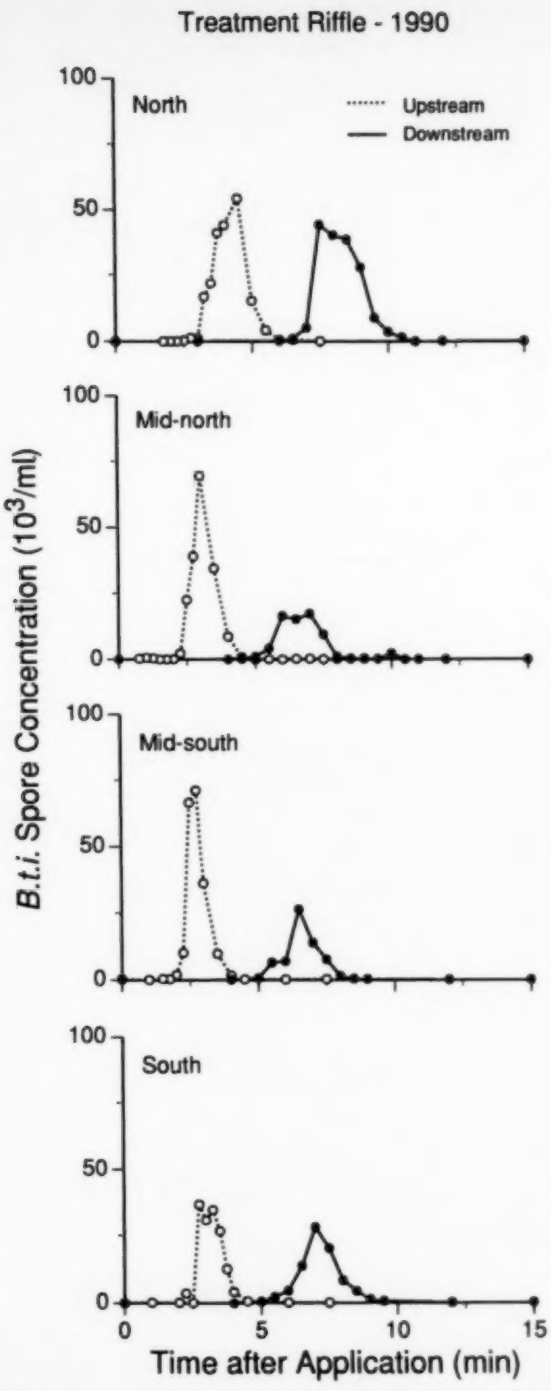

FIG. 4. Concentration of *B.t.i.* spores in water samples from the upstream and downstream sites on each longitudinal sampling transect (i.e., north, mid-north, mid-south, south) in the treatment riffle during the 15 min following the 1990 *B.t.i.* application.

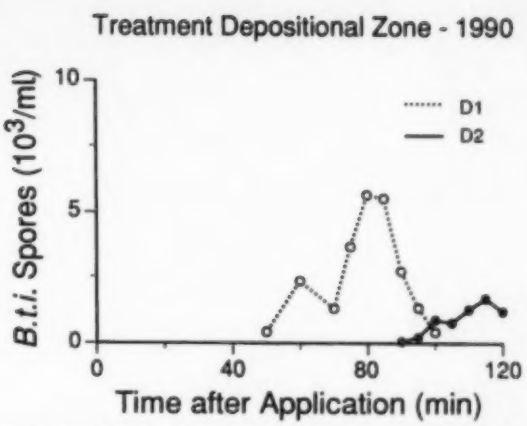

FIG. 5. Concentration of *B.t.i.* spores in water samples from locations D1 and D2 in the treatment depositional zone during the 120 min following the 1990 *B.t.i.* application.

following spraying (mean = 905 spores/cm^2, range = 314–2639 spores/cm^2), indicating some retention of spores in the gravel of the treatment riffle. Spores were also detected at low densities (19 and 100 spores/cm^2) at only two of 10 sampling locations in the depositional zone. Pools were not sampled in 1990.

Low densities of spores (18, 71 and 1129 spores/cm^2) were detected in subsamples from three of five gravel samples collected from the treatment riffle before *B.t.i.* application in 1990; no spores were found when an additional subsample from each of these samples was examined. Densities following *B.t.i.* application increased at these locations to 376, 620, and 2639 spores/cm^2, respectively. A low density of FA-stained spores (69 spores/cm^2) was also observed at one of five sampling locations in the control riffle after *B.t.i.* application in 1990. The presence of FA-stained spores in a few prespray samples from the treatment riffle and one postspray sample from the control riffle suggests either that cross-reactions with other spore-forming bacteria gave false positive responses or that *B.t.i.* had been applied (e.g., accidently by aerial drift) to both riffles at some time prior to the 1990 experiment. Finding spores in postspray gravel samples from the treatment riffle at densities that were generally several times greater than in the prespray samples is consistent with the loss of spores from the water column in transit through the riffle.

In summary, most *B.t.i.* spores were transported through the treatment reach (i.e., 1.5 km of river) immediately following application, indicating that carry in the Susquehanna River was long relative to carry in smaller streams (e.g., Undeen and Lacey 1982; Gaugler et al. 1983). Indirect evidence based on black fly mortality also indicated long carry of *B.t.i.* in large rivers (Lacey et al. 1982; de Moor and Car 1986). This suggests that *B.t.i.* transport between riffle areas in the Susquehanna River is great enough to obviate the need to apply *B.t.i.* to all riffle areas. This would substantially reduce the cost of the black fly control program and the potential impact of residual *B.t.i.* from upstream treatments. Of the spores retained in the treatment reach, low densities accumulated in downstream pools and, to a lesser degree, in the gravel under cobbles in the treatment riffle (spores were not detected in epilithic samples) and possibly in depositional zones along the river's edge. The active filtration of water passing through the riffle by black flies (and pos-

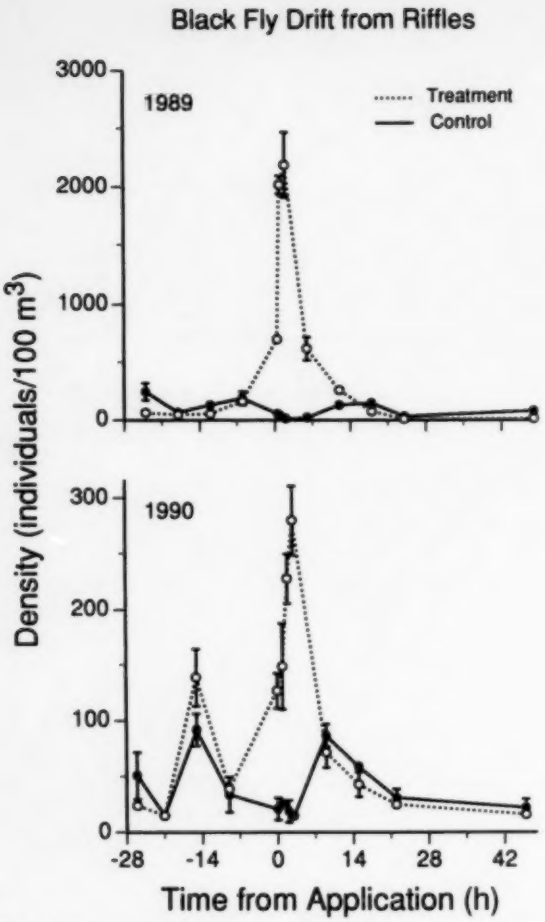

FIG. 6. Density (arithmetic $\bar{X} \pm 1$ SE) of black fly larvae (primarily *Simulium*) in drift below the control and treatment riffles during the 24- to 26-h period preceding and 46- to 48-h period following *B.t.i.* applications in 1989 and 1990. Posttreatment sample times were contrasted with corresponding pretreatment sample times (in 1989: 0.5 vs. −24, 1 vs. −24, 2 vs. −24, 6 vs. −18, 12 vs. −12, 18 vs. −6, 24 vs. −24, and 48 vs. −24 h; in 1990: 0.5 vs. −21, −26, 1 vs. −21, −26, 2 vs. −21, −26, 3 vs. −21, −26, 9 vs. −15, 15 vs. −9, 22 vs. −26, and 46 vs. −26 h). Differences were significant ($P<0.05$) for posttreatment time periods 0.5–12 h in 1989 and 0.5–3 h in 1990.

sibly other filter-feeders) also contributed to the loss of spores from the water column.

Response of Macroinvertebrate Drift to the *B.t.i.* Application

Black fly drift in 1989 and 1990

Drifting black flies (*Simulium*) were common (47–247 individuals/100 m^3 in 1989 and 15–139 individuals/100 m^3 in 1990) during preapplication periods (Fig. 6). Black fly drift in the treatment riffle increased significantly relative to the control riffle within 0.5 h following the *B.t.i.* application and remained high for ≥12 h in 1989 and ≥3 h in 1990. No significant differences were observed during later sampling periods. In these and other contrasts below, significant

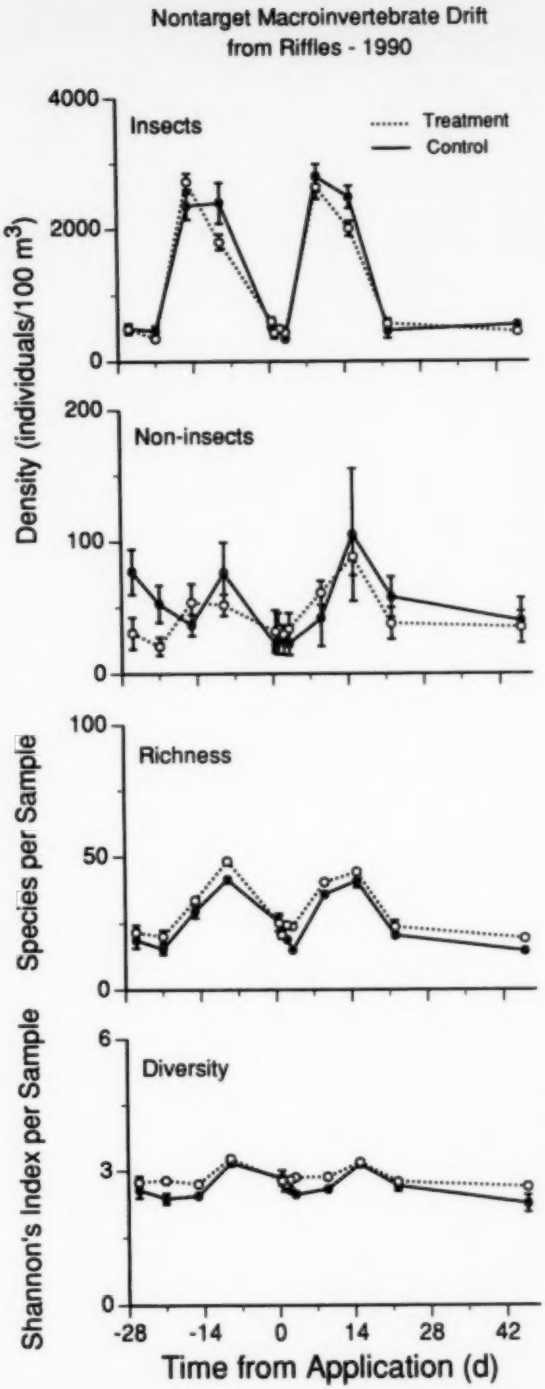

FIG. 7. Total insect density, total noninsect density, species richness, and Shannon's species diversity (arithmetic $\bar{X} \pm 1$ SE) for drift below the control and treatment riffles during the 26-h period preceding and 46-h period following the *B.t.i.* application in 1990. Simuliidae were not included in these estimates. Contrasts of pre- and posttreatment sample times as in Fig. 6; no significant differences were observed.

changes are relative to differences between the treatment and control riffles observed during the corresponding preapplication period. The response of black flies to the *B.t.i.* application was so great that the nonsynchronous sampling in 1989 did not confound data interpretation.

Maximum drift density following the *B.t.i.* application was almost eight times greater in 1989 than in 1990 (2188 versus 281 black flies/100 m^3, respectively) (Fig. 6). This difference in drift density may be a consequence of lower *B.t.i.* exposure (Table 1) and lower benthic densities of black flies in the treatment riffle in 1990. Preapplication drift densities in the control and treatment riffles support the hypothesis that black flies were less abundant in 1990 compared with 1989.

Nontarget macroinvertebrate drift in 1990

Drift data for nontarget macroinvertebrates were analyzed from only 1990 because nonsynchronous sampling in 1989 confounded data interpretation. Macroinvertebrate drift densities were high before the *B.t.i.* application, especially at night (i.e., 1850–2775 individuals/100 m^3 at night versus 367–572 individuals/100 m^3 during the day (Appendix 1)). Ephemeroptera were the most abundant insects in the drift. Diptera and Trichoptera were also common. Noninsects (e.g., Oligochaeta and Gastropoda) comprised <10% (and commonly <3%) of the drifting macroinvertebrates collected (Appendix 1).

Response of nontarget macroinvertebrates within 3 h of the B.t.i. *application*

Unlike black flies, drift of most nontarget macroinvertebrates appeared to be unaffected by *B.t.i.* during the first 3 h following the *B.t.i.* application. Contrasts of total macroinvertebrate abundance, total insect abundance, total noninsect abundance, species richness, and species diversity from before and after the *B.t.i.* application were not significant (Fig. 7; Appendix 1). Changes in drift of individual taxa during this period were significant for only five taxa (*Leucrocuta* (0.5 h), *Anthopotamus* (0.5 h), *Rheotanytarsus* (3 h), *Polypedilum* (0.5, 1, 2, and 3 h), and Leptoceridae (2 h); Appendix 1). With the exception of *Polypedilum*, the above changes appear to represent variations in diel patterns that are unrelated to the *B.t.i.* application because drift increased only briefly (e.g., <1 h for *Leucrocuta* and *Anthopotamus*) compared with the response of black flies.

Drift of *Polypedilum* in the treatment riffle increased for at least 3 h following the *B.t.i.* application. It is difficult to attribute definitively the increase in drift of *Polypedilum* to the *B.t.i.* application because preapplication drift densities and patterns differed markedly between the control and treatment reaches (cf. black flies); in addition, benthic densities were not significantly lower 1 d following the *B.t.i.* application in the treatment riffle or depositional zone (see below).

Response of nontarget macroinvertebrates 6–48 h after the B.t.i. *application*

While black fly drift in the treatment riffle returned to preapplication levels during the 6–48 h following the *B.t.i.* application, significant differences were observed for eight nontarget taxa (*Stenonema* (9 h), *Heptagenia* group (46 h), *Hexagenia* (15 h), *Anthopotamus* (22 h), *Polypedilum* (22 h), *Dicrotendipes* (15 h), *Cricotopus/Orthocladius* (46 h), and *Petrophila* (22 and 46 h); Appendix 1). Drift for two groups (*Stenonema* and *Cricotopus/Orthocladius*) decreased in the treatment riffle whereas densities for the other six groups (*Heptagenia* group, *Hexagenia*, *Anthopotamus*, *Polypedilum*, *Dicrotendipes*, and *Petrophila*) increased in the treatment riffle relative to the control riffle. With the exception of *Petrophila*, these differences were temporally limited to a single sample period, the drift patterns differed markedly from that of black flies, and corresponding changes in benthic densities were not observed (see below). It seems most plausible that these differences reflect naturally occurring spatial/temporal variation in the diel drift patterns for these taxa rather than suggesting a negative effect of *B.t.i.* Increased drift of *Petrophila* at 22 and 46 h may be related to the *B.t.i.* application because it corresponds well with decreased benthic density observed in the treatment riffle 1 and 7 d after the *B.t.i.* application in 1989 (see below).

Drift data from the Susquehanna River clearly support the hypothesis that simuliid larvae are far more sensitive to *B.t.i.* than other river-inhabiting macroinvertebrates. Black fly drift in the treatment riffle increased soon after the *B.t.i.* application and remained high for several hours. These results are similar to results from small and medium-sized streams (e.g., Burton 1984; Gibbs et al. 1986), as well as large rivers (e.g., Lacey et al. 1982; de Moor and Car 1986). In contrast, drift of only two (*Polypedilum* and *Petrophila*) of the 41 nontarget macroinvertebrate taxa examined may have increased in response to the *B.t.i.* application. Other studies of the effects of *B.t.i.* on nontarget macroinvertebrates in streams and rivers have reached contradictory conclusions. Increased drift following the application of *B.t.i.* has been reported for Blephariceridae (Back et al. 1985), two mayflies (*Epeorus fragilis* Morgan and *Baetis brunneicolor* McDunnough), and two caddisflies (*Parapsyche apicalis* (Banks) and *Pycnopsyche divergens* (Walker)) (Pistrang and Burger 1984). Interpretation of these results has been difficult because controls or replication were inadequate or unusually high *B.t.i.* concentrations were used. In contrast, Burton (1984), Car and de Moor (1984), Gibbs et al. (1986), Merritt et al. (1989), and Molloy (1990) generally concluded that *B.t.i.* had no significant effect on the drift of nontarget macroinvertebrates. Unfortunately, none of these drift studies examined *Polypedilum* (most identified midges only to family or subfamily) or *Petrophila*, which would have facilitated comparison with our observations from the Susquehanna River.

Response of Benthic Macroinvertebrate Densities to *B.t.i.* Application

Black flies in riffles (1989) and depositional zones (1990)

Benthic densities of black flies (primarily *Simulium*) decreased to near zero in the treatment riffle 1 d after the *B.t.i.* application (Fig. 8). This corresponds well with the marked increase in drift of black flies from the treatment riffle following the *B.t.i.* application (see above) and the well-documented susceptibility of black flies to *B.t.i.* (i.e., black fly mortality <300 m below an application site is frequently >90% (e.g., Colbo and Undeen 1980; Molloy and Jamnback 1981; Colbo and O'Brien 1984; Back et al. 1985; Gibbs et al. 1986; Merritt et al. 1989; Molloy 1992)). The numerous black flies in the treatment riffle on Day 7 indicates that a single *B.t.i.* application does not have a long-term effect on black flies (Fig. 8). Other studies have observed recruitment of black fly larvae within 1–7 d following a

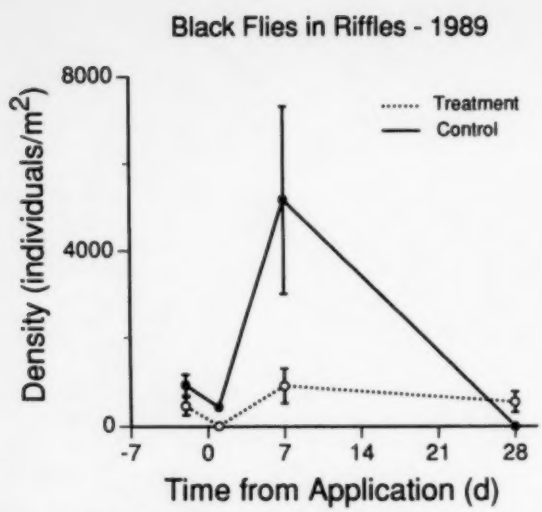

FIG. 8. Density (arithmetic $\bar{X} \pm 1$ SE) of black fly larvae (primarily *Simulium*) in benthos of control and treatment riffles 2 d before and 1, 7, and 28 d after the *B.t.i.* application in 1989. Posttreatment sample dates were contrasted with pretreatment sample date (Day 1 vs. Day −2, Day 7 vs. Day −2, and Day 28 vs. Day −2); differences were significant ($P < 0.05$) on Days 1 and 28.

B.t.i. application (e.g., Colbo and Undeen 1980; Car and de Moor 1984; Back et al. 1985; but not Molloy and Jamnback 1981). Significantly lower benthic densities of black flies in the control riffle on Day 28 may be a response to lower discharge (mean = 56.9 m^3/s for Day −2 through Day 7 versus 21.7 m^3/s on Day 28) that reduced flow through the control riffle and lowered the suitability of that area for black flies. Parallel changes were not observed in the treatment riffle because riverbed morphology maintained adequate flow through the treatment riffle.

No black flies were collected before or after the *B.t.i.* application in the depositional zones sampled in 1990.

Nontarget macroinvertebrates in riffles, 1989

Nontarget macroinvertebrates were very abundant in both riffles. Mean macroinvertebrate density increased from 16 164–19 086 individuals/m^2 at the beginning of the study to 31 641–34 133 individuals/m^2 at the end of the study (Appendix 2). Ephemeroptera were the most abundant insects in the benthic samples, representing 32–55% of total macroinvertebrate densities. Diptera (excluding black flies), Trichoptera, and Coleoptera comprised 9–18, 12–18, and 4–11% of total macroinvertebrate densities, respectively. Noninsects (e.g., Oligochaeta and Planariidae) represented 15–24% of total macroinvertebrate density in most samples.

Response of nontarget macroinvertebrates in the treatment riffle 1, 7, and 28 d after the B.t.i. *application*

Contrasts of total macroinvertebrate abundance, total insect abundance, total noninsect abundance, species richness, and species diversity from before and after the *B.t.i.* application were not significant (Fig. 9; Appendix 2). Contrasts of densities from Days −2 and 1 indicated significant differences for only five individual taxa (unidentified Baetidae, *Anthopotamus*, *Stenelmis*, *Petrophila*, and Planariidae; Appendix 2). Density of unidentified Baetidae was greater in the

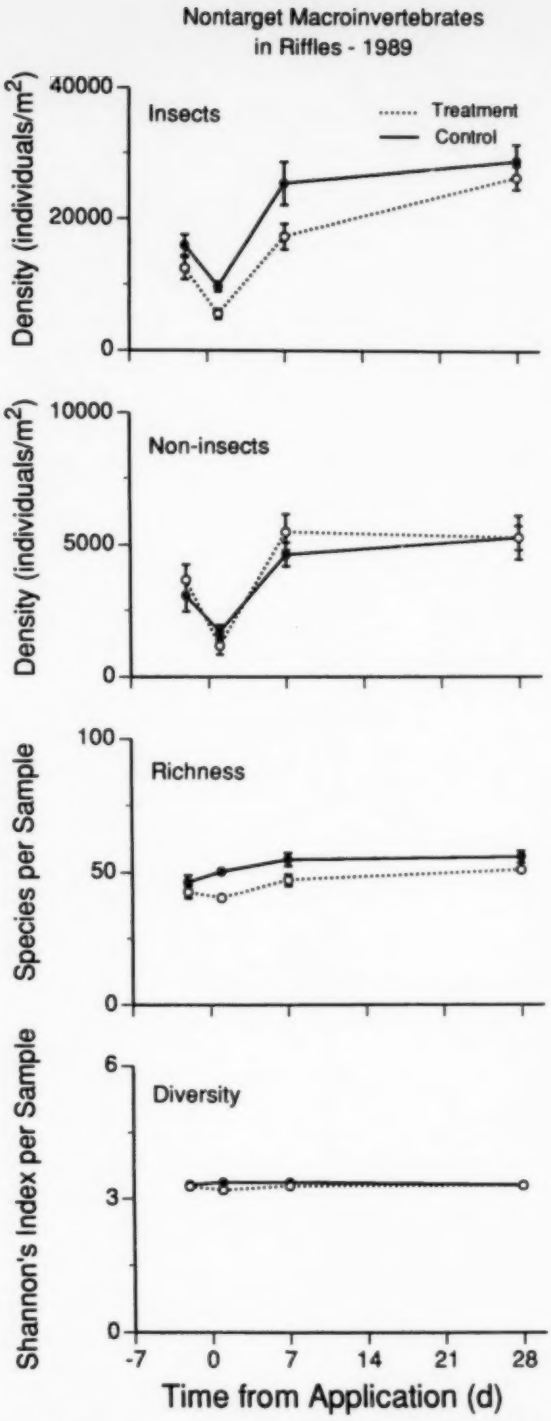

FIG. 9. Total insect density, total noninsect density, species richness, and Shannon's species diversity (arithmetic $\bar{X} \pm 1$ SE) in benthos of control and treatment riffles 2 d before and 1, 7, and 28 d after the *B.t.i.* application in 1989. Simuliidae were not included in these estimates. Contrasts of pre-and posttreatment sample dates as in Fig. 8; no significant differences were observed.

treatment riffle relative to the control riffle on Day 1; densities for the other four groups were lower in the treatment riffle relative to the control riffle on Day 1. The slight increase in density of unidentified Baetidae does not appear to reflect a negative response to the *B.t.i.* application. Changes for *Anthopotamus*, *Stenelpis*, and Planariidae may also be unrelated to the *B.t.i.* application because *B.t.i.* did not appear to affect benthic densities of these taxa in the depositional zone where they were common in 1990 (see below). Moreover, a corresponding increase in drift from the treatment riffle was not observed. A brief increase in drift of *Anthopotamus* occurred 0.5 h after the 1990 *B.t.i.* application, but this response was attributed to natural variation (see above). This brief increase does not appear great enough to produce the observed change in benthic density. In addition, no significant increase in mortality of *Anthopotamus* was observed in response to *B.t.i.* in laboratory bioassays (ANSP 1991).

A negative effect of *B.t.i.* on the aquatic moth *Petrophila* is suggested by the significant reduction in benthic densities on Day 1 and the corresponding increase in drift 22 and 46 h after application (Appendices 1 and 2). Although a toxic effect of *B.t.i.* on *Petrophila* has not been previously reported to our knowledge, a number of subspecies of *Bacillus thuringiensis* (including *B.t.i.*, Chilcott et al. 1990) are at least somewhat toxic to terrestrial Lepidoptera (Cherwonogrodzky 1980). Further experiments are needed to confirm the causal relationship between the *B.t.i.* application and the observed changes in densities and drift of *Petrophila*. In any event, this negative response for *Petrophila* appears short lived, as recolonization returned differences between control and treatment riffles to approximately pre-application conditions by Day 28.

Contrasts of densities from Days −2 and 7 indicated changes in benthic densities for two nontarget macroinvertebrates (*Chimarra* and *Petrophila*; Appendix 2). Density of *Chimarra* was greater in the treatment riffle on Day 7 versus Day −2 whereas density of *Petrophila* was lower in the treatment riffle. Greater densities on Day 7 and the absence of significant contrasts between Days −2 and 1 suggest that *B.t.i.* did not have a negative effect on *Chimarra*. The lower density of *Petrophila* supports the above hypothesis of a negative effect of *B.t.i.* on *Petrophila*.

Benthic densities of eight nontarget macroinvertebrates (*Pseudocloeon*, *Heterocloeon*, unidentified Baetidae, *Tricorythodes*, *Hydropsyche* spp., unidentified Leptoceridae, *Tanytarsus*, and *Thienemannimyia* group) differed significantly on Day 28 compared with Day −2 (Appendix 2). Interpretation of these contrasts was complicated by recruitment that occurred for many taxa between Days −2 and 28. In the control riffle, densities of five groups (*Tricorythodes*, *Hydropsyche* spp., unidentified Leptoceridae, *Tanytarus*, and *Thienemannimyia* group) increased or were relatively unchanged whereas densities of *Pseudocloeon*, *Heterocloeon*, and unidentified Baetidae decreased during this time period. Densities for all eight groups in the treatment riffle on Day 28 were similar to or greater than densities in the treatment riffle on Day −2. These significant contrasts do not appear to reflect a long-term negative effect of a single *B.t.i.* application because in no case did abundance in the treatment riffle decrease while increasing or remaining unchanged in the control riffle and because none of these taxa exhibited significant changes in density on Day 1 or 7. Rather, these changes appear to be the consequence of differential recruitment rates between the treatment and control riffles.

None of the increased densities observed in the treatment riffle was interpreted as a positive response to the removal of black flies by *B.t.i.* This was because black flies occupy a somewhat unique microhabitat (on the upper surfaces of cobbles in fast-flowing water) that is shared with a limited number of macroinvertebrate species, and there is little evidence that black flies competitively exclude these taxa (Crosskey 1990; but see Wiley and Kohler 1981; Dudley et al. 1990). Rather, black fly densities may actually be reduced through competition for space or predation from co-occurring stoneflies, caddisflies, and midges (e.g., Wiley and Kohler 1981; Hemphill 1988, 1991; Hershey and Hiltner 1988; Crosskey 1990; Davies 1991). In addition, because black flies represented such a small proportion of the macroinvertebrate assemblage (<3% of total macroinvertebrate density and <13% of filter-feeder density in the treatment riffle), their brief removal presumably did not markedly increase food available to the macroinvertebrate species that increased in abundance.

Nontarget macroinvertebrates in depositional zones, 1990

Macroinvertebrates were common in the depositional zones (5395–7891 and 7668–11 637 individuals/m^2 in control and treatment zones, respectively), although not as abundant as macroinvertebrates collected in the riffle areas in 1989 (Appendix 2). Diptera and Ephemeroptera were the dominant insect groups in the depositional zones, accounting for 23–35 and 18–32% of total macroinvertebrate density, respectively. Coleoptera, Trichoptera, and Plecoptera were also present (range = 8–14, <1–3, and ≤1% of total macroinvertebrate densities, respectively). Noninsects (Oligochaeta, Planariidae, and Bivalvia) comprised 21–37% of the macroinvertebrates collected.

Response of nontarget macroinvertebrates in the treatment depositional zone 1, 7, and 28 d after the B.t.i. *application*

Contrasts of total macroinvertebrate abundance, total insect abundance, total noninsect abundance, species richness, and species diversity from Days −2 and 1, Days −2 and 7, and Days −2 and 28 indicate that the *B.t.i.* application had no significant effect on these five variables (Fig. 10; Appendix 2). Similarly, there is no indication that *B.t.i.* had a significant impact on benthic densities of individual taxa in the depositional zone (Appendix 2), including several taxa (e.g., Planariidae, *Anthopotamus*, and *Stenelmis*) that decreased in abundance in the riffle.

Field studies that have detected changes in benthic densities of nontarget macroinvertebrates in streams following the application of *B.t.i.* have generally attributed most of these changes to natural processes such as pupation or emergence (Colbo and Undeen 1980; Burton 1984; Car and de Moor 1984; Pistrang and Burger 1984) or sampling error (Colbo and Undeen 1980; Molloy and Jamnback 1981; Burton 1984) rather than to *B.t.i.* It has been suggested that selected chironomids (especially filter-feeding species) in streams and rivers may be affected by *B.t.i.* (e.g., Gaugler and Finney 1982; Car and de Moor 1984; de Moor and Car 1986). For example, Molloy (1992) found in an extensive series of field trials that only filter-feeding chironomids (*Rheotanytarsus* spp.) were susceptible to *B.t.i.* (mortality averaged

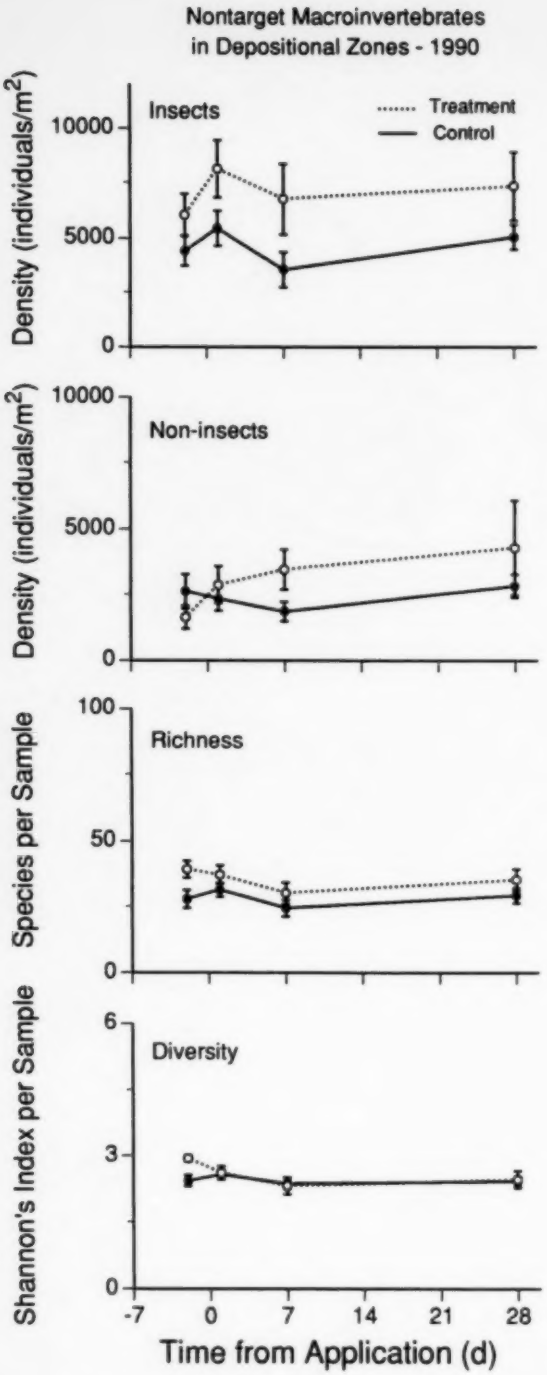

FIG. 10. Total insect density, total noninsect density, species richness, and Shannon's species diversity (arithmetic $\bar{X} \pm 1$ SE) in benthos of control and treatment depositional zones 2 d before and 1, 7, and 28 d after the *B.t.i.* application in 1990. Simuliidae were not included in these estimates. Contrasts of pre- and post-treatment sample dates as in Fig. 8; no significant differences were observed.

23% at sites generally <100 m from the release point), although *B.t.i.* toxicity was far less than for black flies. Similarly, Merritt et al. (1989) found increased mortality for *Rheotanytarsus* 100 m below the point of *B.t.i.* release (37 versus 10% at control), but no effect further downstream (300, 1800, or 2100 m). Back et al. (1985) reported that benthic densities of *Eukiefferiella* and *Polypedilum* (nonfilter-feeding chironomids) were lower (26 and 39%, respectively) 1000 m below the release point of a high concentration of *B.t.i.*, but no differences were observed at 100- or 500-m locations. Of the chironomids examined in our study, only the drift of *Polypedilum* appeared to be negatively affected by *B.t.i.*; the other taxa (nine in the drift and 18 in the benthos, including *Rheotanytarsus*) exhibited no response. That changes in nontarget macroinvertebrate populations in this and previous studies in streams and rivers were relatively small (if at all) and spatially limited compared with those observed for black flies suggests that (1) the overall impact of *B.t.i.* on chironomid and other nontarget macroinvertebrate populations is negligible compared with its effect on black flies and (2) natural variability in benthic densities may have produced some of the significant changes observed for nontarget species (type I errors).

Conclusions

With the exception of *Petrophila* and possibly *Polypedilum*, nonsimuliid macroinvertebrates in the riffle and depositional zone showed no changes in benthic density and drift during the 28 d following the *B.t.i.* application that could be interpreted as negative responses to *B.t.i.* Several taxa exhibited significant changes in field studies that appear related to random error or natural processes such as variation in diel drift patterns or recruitment, and not to the *B.t.i.* application. The results of this study clearly support the hypothesis that *B.t.i.* can be used to suppress black fly populations without negatively affecting most nontarget macroinvertebrates in the Susquehanna River and thus agree with conclusions from studies of smaller lotic systems (e.g., Chilcott et al. 1983; Gibbs et al. 1986; Keenen 1989; Merritt et al. 1989).

There was limited evidence that suggested the reduction in black fly populations in response to *B.t.i.* may have cascaded through the food web and negatively influenced the fish assemblages in the treatment riffle or depositional zone (ANSP 1990, 1991). For example, black flies represented 10–20% of the diet of some fish taxa in the riffle, and black flies represented a significantly smaller proportion in the diets of those species on Days 1 and 7 (R. Horwitz, Academy of Natural Sciences of Philadelphia, 1900 Benjamin Franklin Parkway, Philadelphia, PA 19103, unpublished data). Merritt et al. (1991) suggested similar results for aquatic insect predators that prefer live black flies over other prey; unfortunately, we did not collect the data needed to test this hypothesis in the Susquehanna River. Although dietary changes were observed for some fish species, population structure, physical condition (weight–length relationship), and growth were unaffected for the fish species examined except for a slight reduction in the physical condition of the banded darter (*Etheostoma zonale*) and a 10% reduction in short-term growth rates of the margined madtom (*Noturus insignis*) and spotfin shiner (*Cyprinella spiloptera*, previously *Notropis spilopterus*) (the only two species exam-

ined; ANSP 1990, 1991; R. Horwitz, unpublished data). Furthermore, parallel changes were not observed for these fish taxa in depositional areas, which represent a much greater proportion of the river than riffles. Thus, the potential impact on ecosystem structure and function of using *B.t.i.* to control black flies in the Susquehanna River appears low compared with use of chemical insecticides that have relatively broad toxicities (e.g., Wallace et al. 1973; Helson and West 1978; Flannagan et al. 1979; Yasuno et al. 1982; Sebastien et al. 1989; Dosdall et al. 1991).

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APPENDIX 1. Abundance (arithmetic $\bar{X}$; number/100 m^3) of nontarget macroinvertebrates in drift below riffle habitants before and after the *B.t.i.* application[a] in 1990 and the results of statistical tests[b] used to evaluate differences between time periods 0.5 vs. −21, −26, 1 vs. −21, −26, 2 vs. −21, −26, 3 vs. −21, −26, 9 vs. −15, 15 vs. −9, 22 vs. −26, and 46 vs. −26 h. Simuliidae were not included in these estimates. C = control; T = treatment.

		Drift during sampling periods before and after the *B.t.i* application												
		−26 h	−21 h	−15 h	−9 h	0.5 h	1 h	2 h	3 h	9 h	15 h	22 h	46 h	SE[c]
Total macroinvertebrates	C	572	514	2384	2469	530	515	502	360	2840	2582	520	585	123
	T	521	367	2775	1850	635	461	519	466	2690	2094	602	477	
Ephemeroptera														
Baetidae	C	15	3	137	87	8	2	8	3	190	89	7	4	22
	T	2	8	97	46	4	0	6	11	173	54	8	8	
Baetis	C	0	0	64	70	2	0	0	0	111	67	0	0	12
	T	0	0	115	18	2	2	0	2	140	55	0	0	
Centroptilum	C	6	0	92	17	8	7	10	0	65	48	5	0	11
	T	4	2	187	26	7	0	8	12	95	33	4	9	
Heterocloeon	C	13	23	132	220	56	27	41	16	126	228	70	41	35
	T	58	41	509	247	60	52	69	52	489	222	93	77	
Pseudocloeon	C	12	15	59	95	11	12	14	10	64	114	17	21	14
	T	28	36	135	72	58	34	15	20	102	82	18	13	
Unidentified Baetidae[d]	C	18	14	305	84	11	9	34	29	449	42	10	4	42
	T	41	38	269	103	39	35	43	40	201	75	19	7	
Caenidae														
Caenis														

APPENDIX 1. (*Continued*)

		Drift during sampling periods before and after the *B.t.i* application												
		−26 h	−21 h	−15 h	−9 h	0.5 h	1 h	2 h	3 h	9 h	15 h	22 h	46 h	SE[c]
Ephemerellidae	C	3	3	13	7	4	7	9	0	5	10	0	0	3
Serratella	T	2	5	6	4	7	3	5	0	0	9	2	0	
Heptageniidae	C	0	3	2	6	7	7	7	2	31***	21	0	13	7
Stenonema	T	6	7	47	31	4	0	4	5	22	38	5	6	
Leucrocuta	C	0	0	0	67	4*	0	0	0	2	51	0	0	4
	T	2	2	2	23	0	0	2	1	2	19	0	0	
Stenacron	C	0	0	11	39	0	3	0	0	19	41	0	0	7
	T	0	0	20	27	0	0	2	0	19	23	3	0	
Heptagenia group	C	6	7	15	35	2	6	2	0	12	43	0	0*	6
	T	0	0	37	39	4	2	2	2	58	47	4	7	
Unidentified Heptageniidae[d]	C	21	12	92	50	14	21	21	13	90	67	11	20	15
	T	18	17	139	91	13	13	21	22	137	103	26	24	
Leptophlebiidae	C	0	0	17	147	4	0	0	0	10	131	0	0	9
Choroterpes	T	0	0	4	105	8	0	0	0	9	93	2	0	
Ephemeridae	C	0	0	2	254	0	0	0	0	0	154***	0	0	14
Hexagenia	T	4	8	4	43	4	2	0	2	8	113	2	0	
Oligoneuriidae	C	0	0	19	87	0	2	10	0	31	49	3	0	8
Isonychia	T	0	0	58	110	0	4	6	1	73	90	11	0	
Potamanthidae	C	89	53	695	210	64*	64	85	75	758	363	40*	68	35
Anthopotamus	T	40	25	169	171	72	34	36	47	187	259	57	42	
Leptohyphidae	C	17	15	71	99	17	27	9	16	65	162	18	0	17
Tricorythodes	T	56	9	42	96	24	17	30	16	65	142	38	25	
Plecoptera	C	0	0	0	15	2	8	7	0	2	14	0	0	3
Perlidae	T	7	0	0	7	0	0	0	0	0	9	2	5	
Trichoptera														
Hydropsychidae	C	9	12	10	11	17	2	4	7	11	25	8	8	5
Hydropsyche spp.	T	14	8	9	24	9	7	9	12	13	17	5	7	
Cheumatopsyche	C	0	7	0	6	5	2	2	2	2	2	0	0	3
	T	4	7	6	2	0	2	0	2	2	4	2	7	
Macrostemum	C	0	5	0	3	2	0	0	0	2	5	5	0	2
	T	4	4	2	12	3	2	2	1	6	7	2	0	
Glossosomatidae	C	0	5	2	13	2	0	2	0	0	8	2	12	3
Protoptila	T	4	0	0	3	2	0	0	5	4	2	4	0	
Hydroptilidae	C	19	0	17	16	9	16	21	21	37	46	32	18	9
Hydroptila	T	6	7	11	21	10	7	5	5	4	12	14	11	
Leptoceridae	C	0	0	333	98	4	7	7	8**	270	89	3	8	20
	T	5	9	521	41	2	5	11	2	427	85	4	3	

APPENDIX 1. (*Concluded*)

		Drift during sampling periods before and after the *B.t.i* application												
		−26 h	−21 h	−15 h	−9 h	0.5 h	1 h	2 h	3 h	9 h	15 h	22 h	46 h	SE[c]
Coleoptera														
Elmidae	C	6	0	4	45	9	0	3	5	2	46	2	0	6
Stenelmis	T	4	2	6	52	5	4	2	4	6	39	0	6	
Lepidoptera														
Pyralidae	C	12	0	11	17	5	7	9	6	15	7	0**	0*	5
Petrophila	T	8	11	39	10	15	24	10	18	19	11	24	21	
Diptera														
Chironomidae														
Tanytarsini	C	37	45	50	41	39	61	35	20**	68	72	23	27	16
Rheotanytarsus	T	59	7	30	109	22	52	61	40	66	100	80	44	
Tanytarsus	C	9	3	0	10	0	8	7	0	3	8	8	0	3
	T	2	2	0	0	2	2	2	0	0	2	4	0	
Chironomini	C	60	98	15	220	47***	77*	51*	31*	61	170	46*	103	25
Polypedilum	T	4	4	33	75	81	48	34	37	96	94	50	19	
Dicrotendipes	C	4	0	10	27	0	5	6	0	17	8*	9	0	4
	T	0	2	0	0	2	2	2	2	0	4	0	5	
Pentaneurini	C	0	0	9	0	0	0	0	0	20	6	2	0	4
Thienemannimyia	T	2	0	13	4	0	0	0	1	6	4	0	0	
Thienemannimyia group	C	0	0	12	38	0	0	0	0	15	25	2	0	6
	T	2	0	15	15	4	2	0	4	20	15	2	0	
Ablabesmyia	C	0	0	22	2	0	0	2	5	20	12	3	0	3
	T	0	0	2	4	0	0	0	0	3	8	0	3	
Nilotanypus	C	0	0	0	3	7	4	2	0	3	0	3	0	3
	T	0	2	7	8	6	2	2	4	2	6	2	2	
Orthocladiini	C	12	10	2	13	7	2	6	7	12	5	0	9	5
Tvetenia	T	21	11	21	13	8	14	27	12	7	18	8	5	
Cricotopus/Orthocladius sp. 2	C	66	60	68	100	65	46	39	40	75	109	92	125***	28
	T	49	22	57	35	57	19	31	17	42	36	30	46	
Planariidae	C	0	0	0	0	0	3	0	0	0	0	3	0	2
	T	4	0	8	2	0	0	0	0	0	2	0	3	
Oligochaeta	C	45	16	12	17	8	6	17	0	2	36	27	19	10
	T	8	6	13	7	16	16	6	16	9	18	14	15	
Acari	C	12	8	7	0	4	4	4	6	13	3	3	4	4
	T	11	6	12	4	6	9	4	4	14	2	11	14	
Gastropoda	C	16	26	7	28	4	13	4	15	8	50	17	12	9
	T	0	7	21	15	3	4	9	8	13	33	7	4	
Bivalvia	C	0	3	4	15	5	0	0	2	6	3	5	0	3
	T	6	0	0	17	7	5	10	4	14	18	6	0	

[a]See text for times of sampling periods.
[b]*$P<0.05$; **$P<0.01$; ***$P<0.001$.
[c]Pooled standard error equals $(s^2/n)^{1/2}$ where s^2 is the sample variance of the untransformed data pooled by replicate and date and n (=4) is the sample size.
[d]Unidentified specimens were very small or damaged.

APPENDIX 2. Abundance (arithmetic $\bar{X}$; number/m^2) of nontarget marcroinvertebrates in riffle and depositional habitats 2 d before and 1, 7, and 28 d after the *B.t.i.* application and the results of statistical tests[a] used to evaluate differences between Days 1 and −2, Days 7 and −2, and Days 28 and −2 in 1989 and 1990. Simuliidae were not included in these estimates. C = control; T = treatment.

		Riffles in 1989					Depositional zones in 1990				
		Day −2	Day 1	Day 7	Day 28	SE[b]	Day −2	Day 1	Day 7	Day 28	SE[b]
Total	C	19 086	11 402	30 094	34 133	2284	7035	7777	5395	7891	1434
macroinvertebrates	T	16 164	6715	22 852	31 641		7668	11 008	10 227	11 637	
Ephemeroptera											
Baetidae	C	1953	539	1867	891	221	8	78	0	0	48
Baetis	T	641	184	1039	703		27	43	109	63	
Heterocloeon	C	78	59	578	23*	85		NA[c]			
	T	63	16	102	141						
Pseudocloeon	C	1086	500	1648	219***	176	4	16	0	0	39
	T	688	152	1110	922		66	156	4	16	
Centroptilum	C		NA				148	121	47	20	30
	T						102	43	129	27	
Unidentified Baetidae[d]	C	406	105*	641	172***	81	4	39	0	0	15
	T	63	27	398	297		16	20	35	23	
Caenidae	C	484	313	695	805	90	148	238	39	86	102
Caenis	T	469	254	484	414		422	457	160	180	
Ephemerellidae	C	445	238	508	297	81		NA			
Serratella	T	234	66	281	156						
Heptageniidae	C	727	379	828	2148	148	0	39	0	4	85
Stenonema	T	836	527	1141	2211		105	82	66	406	
Leucrocuta	C	414	246	359	297	97	47	82	47	4	49
	T	555	371	773	531		74	129	113	70	
Stenacron	C	0	0	0	86	18	27	23	4	78	34
	T	8	63	16	156		23	8	8	51	
Rhithrogena	C	141	82	86	17	29		NA			
	T	47	8	8	0						
Heptagenia group	C	430	297	383	313	83	4	12	16	4	28
	T	602	441	711	375		78	20	86	63	
Unidentified Heptageniidae[d]	C	219	117	281	336	88	16	39	4	12	43
	T	344	301	227	539		117	109	59	94	
Oligoneuriidae	C	250	262	1242	3898	340		NA			
Isonychia	T	609	699	867	1648						
Leptophlebiidae	C		NA				113	102	55	59	33
Choroterpes	T						86	90	16	39	
Ephemeridae	C		NA				355	590	445	598	107
Hexagenia	T						43	20	4	78	
Potamanthidae	C	2195	1848**	3242	4758	290	426	719	332	414	469
Anthopotamus	T	1242	445	1336	2086		1102	1469	1148	1480	
Leptohyphidae	C	16	23	86	258**	30	301	281	184	70	75
Tricorythodes	T	0	12	31	16		121	102	35	47	

APPENDIX 2. (*Continued*)

		Riffles in 1989					Depositional zones in 1990				
		Day −2	Day 1	Day 7	Day 28	SE[b]	Day −2	Day 1	Day 7	Day 28	SE[b]
Polymitarcyidae	C	117	152	31	0	32		NA			
Ephoron	T	219	133	148	0						
Plecoptera	C	180	125	219	305	48	20	31	12	0	31
Perlidae	T	39	43	133	203		78	47	70	51	
Hemiptera	C		NA				27	35	16	98	29
Corixidae	T						4	8	8	43	
Megaloptera											
Sialidae	C		NA				51	47	8	4	11
Sialis	T						8	16	8	4	
Trichoptera											
Hydropsychidae	C	844	383	781	586*	145		NA			
Hydropsyche spp.	T	625	129	406	1500						
Cheumatopsyche	C	844	516	1219	977	129		NA			
	T	680	195	570	836						
Macrostemum	C	148	102	484	242	146		NA			
	T	570	242	805	1266						
Philopotamidae	C	23	23	305*	1125	103		NA			
Chimarra	T	0	4	31	672						
Glossosomatidae	C	203	90	203	422	76		NA			
Protoptila	T	234	55	289	531						
Hydroptilidae	C	156	90	164	180	43	16	16	4	4	9
Hydroptila	T	23	31	133	70		23	23	4	20	
Leptoceridae	C	31	23	156	211	36	0	0	0	4	6
Ceraclea	T	23	16	141	172		12	12	0	16	
Unidentified Leptoceridae[d]	C	16	0	31	305*	36	8	0	0	8	10
	T	8	12	23	47		20	23	16	35	
Coleoptera											
Elmidae	C	594	375	680	711	70	31	20	0	0	48
Optioservus	T	250	94	398	250		180	121	121	129	
Stenelmis	C	1164	828***	1477	1820	204	82	94	35	39	133
	T	1039	148	1141	1469		262	586	375	207	
Dubiraphia	C		NA				570	766	316	770	257
	T						250	391	781	840	
Unidentified Elmidae[d]	C	148	43	281	188	54	12	0	8	4	20
	T	86	23	78	94		43	59	43	35	
Psephenidae	C	23	31	23	55	18		NA			
Psephenus	T	86	27	86	31						

APPENDIX 2. (*Continued*)

		Riffles in 1989					Depositional zones in 1990				
		Day −2	Day 1	Day 7	Day 28	SE[b]	Day −2	Day 1	Day 7	Day 28	SE[b]
Hydrophilidae	C		NA				63	94	90	86	45
Berosus	T						8	55	55	47	
Lepidoptera											
Pyralidae	C	78	78**	313**	1133	167		NA			
Petrophila	T	367	105	406	3344						
Diptera											
Chironomidae											
Tanytarsini	C	594	445	2023	2242	357	16	39	16	8	111
Rheotanytarsus	T	273	207	1852	3063		39	238	422	309	
Tanytarsus	C	8	0	31	383***	44	422	465	598	805	271
	T	0	4	0	23		957	1199	1344	1113	
Cladotanytarsus	C		NA				43	63	66	16	20
	T						35	47	51	12	
Stempellinella	C	16	4	8	39	9	59	105	105	102	73
	T	0	0	8	31		258	320	148	125	
Chironomini	C		NA				234	320	262	254	141
Chironomus	T						348	449	211	27	
Polypedilum	C	219	227	672	594	101	453	227	156	488	144
	T	250	94	453	430		51	51	215	410	
Ablabesmyia	C		NA				117	59	63	203	24
	T						55	47	31	39	
Cryptochironomus	C		NA				43	16	16	20	12
	T						12	39	31	4	
Cladopelma	C		NA				35	47	12	35	19
	T						23	55	27	31	
Thienemannimyia group	C	0	4	23	156***	15	4	20	8	27	13
	T	23	8	8	16		16	8	4	16	
Thienimannimyia	C	133	94	164	609	51		NA			
	T	63	27	78	125						
Orthocladiini	C	195	82	148	289	52		NA			
Tvetenia	T	195	12	63	367						
Cardiocladius	C	328	78	438	461	71		NA			
	T	109	12	109	383						
Cricotopus/Orthocladius	C	305	406	1367	297	156	0	12	4	0	26
	T	234	98	461	63		74	109	4	0	
Microtendipes	C	8	27	31	125	27	4	12	12	27	13
	T	47	31	63	8		16	27	8	20	
Dicrotendipes	C		NA				125	113	207	195	93
	T						273	469	129	145	
Procladius	C		NA				86	82	23	63	56
	T						12	16	113	195	
Unidentified Chironomidae[d]	C	133	78	180	266	51	117	109	156	121	90
	T	102	59	117	398		184	590	258	313	

APPENDIX 2. (*Concluded*)

		Riffles in 1989					Depositional zones in 1990				
		Day −2	Day 1	Day 7	Day 28	SE[b]	Day −2	Day 1	Day 7	Day 28	SE[b]
Ceratopogonidae	C		NA				31	35	12	66	14
	T						0	0	27	51	
Planariidae	C	1844	711***	3109	3273	281	59	55	59	113	52
	T	1555	125	1672	1789		207	184	90	156	
Nematoda	C	23	35	31	23	19	27	82	20	39	20
	T	86	12	39	31		8	98	51	12	
Oligochaeta	C	938	703	992	1078	283	1988	1719	1402	2023	763
	T	1453	398	1625	1102		844	2082	2398	3309	
Amphipoda	C		NA				105	137	98	98	62
Gammaridae	T						156	98	121	195	
Acari	C	39	16	39	31	14	12	8	8	74	21
	T	39	12	31	16		51	39	27	55	
Gastropoda	C	39	27	39	86	21	39	35	12	266	90
Ancylidae	T	0	12	16	8		51	59	63	90	
Bivalvia	C	188	199	398	734	238	379	293	227	180	122
	T	531	590	2086	2297		277	301	590	453	

[a]$*P < 0.05$; $**P < 0.01$; $***P < 0.001$.
[b]Pooled standard error equals $(s^2/n)^{1/2}$ where s^2 is the sample variance of the untransformed data pooled by replicate and date and n (=8) is the sample size.
[c]NA = not applicable because taxon was absent or densities were too low for analysis.
[d]Unidentified specimens were very small or damaged.

Effects of Dietary Iron Concentrations on the Cytochrome P450 System of Atlantic Salmon (*Salmo salar*)

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Groups of Atlantic salmon (*Salmo salar*) parr were fed a basal diet containing 27 mg Fe·kg feed^{-1} supplemented with different iron levels (0, 80, and 160 mg·kg^{-1}, respectively) for 8 wk. After this period, a subgroup of each iron regime was treated intraperitoneally with β-naphthoflavone (BNF), a potent inducer of cytochrome P450 1A1 in fish. Differences between the iron regimes were observed in hepatic iron content, which was higher in the high-iron group, and in P450 1A1 levels and monooxygenase activity, measured as 7-ethoxyresorufin *O*-deethylase (EROD), which were lower in the high-iron group. Differences were also observed in the induction response of P450 1A1 and EROD to BNF. Levels of a constitutive isozyme denoted P450con, belonging to the P450 3A subfamily, did not respond to the dietary iron in the same manner, demonstrating selective regulatory effects of iron on different P450 isozymes.

Pendant 8 sem, des groupes de tacons de saumon atlantique (*Salmo salar*) ont été soumis à un régime de base contenant 27 mg Fe·kg d'aliments^{-1} avec apports complémentaires de fer à différentes concentrations (respectivement 0, 80 et 160 mg·kg^{-1}). Après cette période, de la β-naphthoflavone (BNF), inducteur puissant du cytochrome P450 1A1 chez le poisson, a été administrée par voie intrapéritonéale à un sous-groupe de chacun des groupes recevant du fer. Des différences entre les apports de fer ont été observées dans la teneur en fer hépatique, qui était plus élevée chez le groupe recevant la dose la plus élevée, et dans les teneurs en P450 1A1 et dans l'activité de la monooxygénase, mesurée sous forme de 7-éthoxyrésorufin *O*-dééthylase (EROD), qui était plus faible dans le groupe recevant une dose élevée en fer. Des différences ont également été relevées au niveau de l'induction du P450 1A1 et de l'EROD par la BNF. Une isozyme constitutive, appelée P450con, appartenant à la sous-famille P450 3A, ne réagissait pas au fer alimentaire de la même façon, ce qui montre que le fer a des effets régulateurs sélectifs sur les différentes isozymes du complexe P450.

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The cytochrome P450 (CYP or P450) gene superfamily encodes many isoforms that are involved in a broad range of chemical transformations, attacking a large number of substrates (Nebert and Gonzalez 1987). These include physiologically important compounds such as steroids, fatty acids, and eicosanoids, and xenobiotics such as drugs, pesticides, carcinogens, and other environmental pollutants. The enzyme system has been intensively studied in mammals in the last decade (Guengerich 1987; Nelson et al. 1993), and current knowledge of the characteristics in fish has been summarized in recent reviews (Stegeman 1989; Stegeman et al. 1990; Goksøyr and Förlin 1992).

A number of factors that affect the P450 system in fish have been identified, including development and age, sexual maturation, temperature and season, species and strain, and the presence of inducing agents (see Andersson and Förlin 1992; Goksøyr and Förlin 1992). Diet and nutrition are known to influence biotransformation activities in mammals (Anderson et al. 1982; Parke 1991; Yang et al. 1992).

In fish, increasing efforts have been made to establish connections between nutrition, immune competence, and disease resistance (Landolt 1989). However, only a few reports have focused on possible connections between dietary status and xenobiotic metabolism and effects in fish (e.g., Ankley et al. 1989). Dietary influences on xenobiotic metabolism may alter the therapeutic effects of drugs and the toxicity or carcinogenicity of environmental chemicals (Parke 1991; Yang et al. 1992). A deeper knowledge of these factors in fish is therefore warranted both from a pharmacological point of view for fish farming and from an ecotoxicological standpoint. The increasing interest in using the P450 response of fish in monitoring the aquatic environment (Goksøyr and Förlin 1992) makes it especially important to be aware of the factors affecting background levels and induction responses.

In our studies of the P450 system of Atlantic salmon (*Salmo salar*), an important aquaculture species, we have

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so far described inducing effects of model xenobiotics (Goksøyr and Larsen 1991) and variations during sexual maturation and spawning (Larsen et al. 1992). In this study, we investigated the effects of different concentrations of dietary iron on the P450 system in juvenile Atlantic salmon parr.

Iron is an essential trace mineral for fish with a suggested dietary requirement of 60 mg·kg dry feed^{-1} in Atlantic salmon (Lall 1989); it is known to induce intracellular lipid peroxidation (Lee et al. 1981), and excess amounts in fish feed are harmful (Desjardins et al. 1987). Iron is present in varying amounts in commercial fish feed, ranging from 50 to 500 mg·kg dry feed^{-1} (Maage 1991). The P450 isozymes are hemoproteins, with iron-protoporphyrin IX as an essential part of the holoenzyme (Goksøyr and Förlin 1992). We examined how different levels of dietary iron affected the expression and inducibility of specific P450 isozymes and catalytic activities in Atlantic salmon.

Materials and Methods

7-Ethoxyresorufin and resorufin were purchased from Pierce Eurochemie (Holland). NADPH, β-naphthoflavone (BNF), 4-chloronaphthol, *o*-phenylenediamine hydrochloride, and sodium dodecyl sulfate (SDS) were from Sigma Chemical Co. (United Kingdom). Equipment, secondary antibodies, and other chemicals for Western blotting and ELISA were obtained from Bio-Rad (United States). All other chemicals were of the highest commercial grade available.

An experimental feed based on a commercial cod muscle meal (TORO A/S, Bergen, Norway) as a protein source was formulated as described by Maage and Julshamn 1993 (Table 1). This feed produces satisfactory growth in Atlantic salmon (Maage and Julshamn 1993). It contains an iron concentration of 27 mg·kg dry feed^{-1} which is far lower than what is found in commercial feed (Tacon and De Silva 1983; Maage et al. 1989). The feed was supplemented with 0, 80, and 160 mg Fe·kg^{-1}, respectively, as $FeSO_4 \cdot 7H_2O$. Iron sulfate was mixed with a small volume of corn meal, which subsequently was mixed with the other ingredients. The feed was cold-pelleted through a 2.5-mm die and dried before feeding. These diets, containing a total of 33, 101, and 190 mg Fe·kg dry feed^{-1} by analysis, will be referred to as low-, medium-, and high-iron-containing diets, respectively.

Juvenile Atlantic salmon weighing 31.6 ± 6.0 g were fed the experimental diet without iron supplementation for 14 d to acclimate to this feed. The fish were then divided into tanks of 1 × 1 m, about 90 fish in each tank, and duplicate groups were fed each of the prepared diets for 8 wk before sampling. The fish were fed using automatic feeders feeding for 20 s every 7 min. The feeding rate followed the ratios proposed by Austreng et al. (1987). The feeding experiments were performed at the Aquaculture Research Station at Matre.

Fish were kept in fresh water supplemented with 1–2% seawater from a seawater well to increase the conductivity and to buffer the acidity of the extremely soft fresh water found in Western Norway (Maage et al. 1989). The water temperature was recorded daily and followed the ambient water temperature of the Matre River, i.e., 9°C at the start of the experiment, falling to 8°C after 4 wk and 7°C after 8 wk. The elemental analysis of the rearing water showed 13.5–33.5 μg Fe·L^{-1}, 5–17 mg Ca·L^{-1}, and 15–43 mg Mg·L^{-1}. The variation in the elemental concentrations in the water was caused by fluctuations in the salinity of the supplemental seawater.

Fish from each of the different feeding groups were taken live back to the laboratory. Liver samples of four fish from each group were taken on arrival, and microsomes were prepared as previously described (Goksøyr and Larsen 1991). At the same time, five fish in each group were treated intraperitoneally (ip) with 50 mg BNF·kg body weight^{-1}, dissolved and sonicated in soybean oil (10 mg BNF·mL).$^{-1}$ They were kept in glass tanks in aerated water from Matre at 5°C for 5 d after injection. One fish in the low-iron group and two fish in the high-group were lost when they jumped out of the tanks. After 5 d, the rest of the fish were killed, livers were excised, and microsomes were prepared.

Analyses of iron concentrations were performed on a Perkin-Elmer 3030 flame atomic absorption spectrophotometer. For details on preparation of samples and performance of analyses, see Maage et al. (1989). Measurements of protein, cytochrome P450, NADPH-cytochrome P450 reductase, 7-ethoxyresorufin *O*-deethylase (EROD), and immunochemical techniques (Western blotting and P450 1A1-ELISA) were performed at optimal conditions for Atlantic salmon as previously described (Goksøyr 1991; Goksøyr and Larsen 1991; Larsen et al. 1992).

Results were log-transformed before statistical analyses. Differences between feeding groups were analyzed by an ANOVA. Effects of BNF treatment were analyzed by a *t*-test between the corresponding control and BNF groups. Alpha level was set at $p < 0.05$. Statistical analyses were performed using the JMP Software for Statistical Visualization (SAS Institute Inc.) on an Apple Macintosh.

Results

Growth and Hepatic Iron Content

The fish grew to 38 ± 8, 38 ± 8, and 39 ± 9 g in the low-, medium-, and high-iron groups, respectively, with no mortality in the experimental period. There were no significant differences in growth between the feeding regimes, indicating similar feed preference in the three groups. However, hepatic iron concentrations were different (ANOVA, $p < 0.05$) between regimes: 75 ± 30, 96 ± 24, and 118 ± 35 mg Fe·kg wet weight^{-1} in the low-, medium-, and high-iron diets, respectively.

Cytochrome P450 System Related to Feeding Regimes

Total cytochrome P450 was determined spectrophotometrically in pooled samples from each group only; thus, no statistics could be performed, and differences between feeding groups are not evident (Table 2). Basal NADPH-cytochrome P450 reductase was not affected by the feeding regime. However, the cytochrome P450 dependent EROD activity was significantly depressed in the high-iron group (Table 2).

Of the two isozymes measured immunochemically by indirect ELISA (results given as semiquantitative absorbance values related to protein), P450 1A1 (detected by anti-cod P450 1A1 IgG) was affected by the feeding regime whereas P450con cross-reacting protein (detected by anti-rainbow trout P450con IgG and recently demonstrated to belong to the

TABLE 1. Composition of the basal experimental diet.

Ingredient	g·kg^{-1}
Cod muscle meal[a]	610
Capelin oil[b]	180
Corn meal[c]	150
Mineral mixture[d]	50
Vitamin mixture[e]	10

[a]From TORO A/S, Norway.
[b]NORSALMOIL, from NORDSILDMEL A/S, Norway.
[c]CODRICO LTD., The Netherlands.
[d]The mineral mixture supplied the following minerals (as mg·kg dry diet^{-1}): Ca (as $CaHPO_4 \cdot 2H_2O$), 4300; P (as $CaHPO_4 \cdot 2H_2O$ and KH_2PO_4, 50/50), 7000; K (as KH_2PO_4), 4000; NaCl, 10 000; Mg (as $MgSO_4$), 1000; Zn (as $ZnSO_4 \cdot 7H_2O$), 100; Mn (as $MnSO_4 \cdot 4H_2O$), 25; Cu (as $CuSO_4 \cdot 5H_2O$), 5; I (as KI), 11.
[e]The vitamin mixture supplied the following vitamins (as mg·kg dry diet^{-1}, unless otherwise noted): thiamin, 10; riboflavin, 10; pantothenic acid, 10; niacin, 20; pyridoxin, 40; biotin, 0.5; folic acid, 20; vitamin B_{12}, 0.2; inositol, 500; ascorbate polyphosphate, 1000; choline chloride, 5500; menadione, 50; BHT, 25; retinyl palmitate, 7000 IU·kg^{-1}; cholecalciferol, 3000 IU·kg^{-1}; alpha-tocopherol, 200 IU·kg^{-1}.

CYP 3A subfamily; M. Celander and J.J. Stegeman, personal communication) was not (Table 2). Similar to EROD activities, P450 1A1 levels were lowest in the high-iron group. Correspondingly, individual EROD activities and P450 1A1 absorbance levels (both log-transformed) were well correlated in all feeding groups ($p < 0.05$, $r = 0.91$; Fig. 1).

Effects of BNF Treatment

Total cytochrome P450 (measured in pools) was slightly elevated in the high-iron group (0.69 versus 0.6 nmol P450·mg protein^{-1}) after BNF treatment whereas no effect could be seen in the other groups (Table 2).

NADPH-cytochrome P450 reductase was significantly induced only in the low-iron group, with a 100% increase in activities. This group was also significantly different from the other BNF-treated groups (Table 2).

EROD activities were significantly induced by BNF in all feeding groups, but the final activity was higher in the low-iron group compared with the others (borderline significance in ANOVA, $p = 0.063$). The high-iron group, which had the lowest basal activities, was induced to the same final activity as the medium-iron group (Table 2).

P450 1A1 was also significantly induced in all feeding groups, but with no difference in final levels between groups. P450con cross-reacting protein was slightly depressed in the medium-, and high-iron groups. But whereas this effect was not significant in a pairwise comparison of control and BNF groups, the changes induced significant differences between the different BNF groups, with the low-iron group being significantly higher in P450con levels than the two other groups (Table 2).

In the BNF-treated individuals, a very strong correlation was seen between EROD and NADPH-cytochrome P450 reductase activities ($r = 0.97$, $p < 0.00005$), but as opposed to in the control groups, no correlation was observed between EROD and P450 1A1 level (Fig. 2).

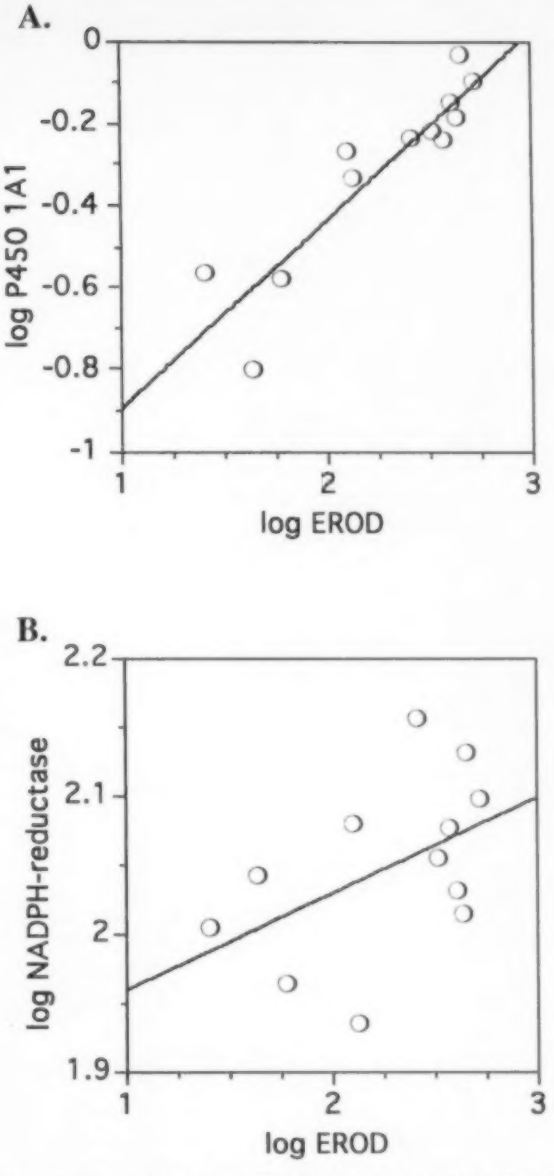

FIG. 1. Correlation plots of EROD activities with (A) P450 1A1 protein levels (measured as ELISA Abs.) and (B) NADPH-cytochrome P450 reductase (NADPH-reductase) activities in liver microsomes from Atlantic salmon given different dietary levels of iron (control group). Individual data are log-transformed. Linear curve fits are represented by the lines (A) $f(x) = 0.458 \cdot x - 1.352$ ($R^2 = 0.829$) and (B) $f(x) = 0.069 \cdot x + 1.890$ ($R^2 = 0.233$).

Discussion

We have demonstrated that the P450 system of Atlantic salmon responds to different iron levels in the diet. Differences were observed in the levels of EROD activities and P450 1A1 isozyme content, as well as in the response to BNF of all parameters (EROD, NADPH-cytochrome P450

TABLE 2. Effects of dietary iron on cytochrome P450 parameters and induction response in liver microsomes from Atlantic salmon before and after treatment with BNF. *Significantly different from the corresponding control group ($p < 0.05$).

Parameter	mg Fe added·kg^{-1}[a] 0	80	160	ANOVA[b]
Control				
N	4	4	4	—
ΣP450 (nmol·mg^{-1})[c]	0.64	0.56	0.60	—
NADPH-reductase (nmol·min^{-1}·mg^{-1})	113 ± 22	120 ± 17	106 ± 12	0.5689
EROD (pmol·min^{-1}·mg^{-1})	386 ± 174	355 ± 72	64 ± 44	0.0012
P450 1A1 (ELISA Abs.)	0.72 ± 0.20	0.60 ± 0.03	0.31 ± 0.16	0.0088
P450con (ELISA Abs.)	0.95 ± 0.17	0.93 ± 0.19	0.87 ± 0.32	0.809
BNF				
N	4	5	3	—
ΣP450 (nmol·mg^{-1})[c]	0.64	0.58	0.69	—
NADPH-reductase (nmol·min^{-1}·mg^{-1})	236 ± 119*	104 ± 9	123 ± 34	0.0392
EROD (pmol·min^{-1}·mg^{-1})	3221 ± 1724*	1548 ± 247*	1933 ± 359*	0.0630
P450 1A1 (ELISA Abs.)	1.56 ± 0.04*	1.32 ± 0.24*	1.37 ± 0.51*	0.4824
P450con (ELISA Abs.)	1.01 ± 0.09	0.70 ± 0.10	0.71 ± 0.23	0.0296

[a]Supplemented to a basal diet containing 27 mg Fe·kg dry feed^{-1}.
[b]Effects of diet; *p*-values obtained from ANOVA analyses.
[c]Measured in pooled samples; mean of parallell analyses.

reductase, P450 1A1, and P450con). In the same feeding experiment, no effects of iron were seen in other organs or tissues (Bjørnevik and Maage 1993), with the exception of increased accumulation of iron in the liver in the higher iron dose groups.

The question of how dietary iron affects drug metabolism has usually been studied from the side of iron deficiency in mammals (see Parke and Ioannides 1981; Dhur et al. 1989; Yang et al. 1992). Chronic iron deficiency generally leads to stimulation of some P450 activities whereas others remain unchanged (Parke and Ioannides 1981; Dhur et al. 1989; Yang et al. 1992).

In our experiment, no sign of iron deficiency (measured by hematological parameters such as hemoglobin and hematocrit levels) was observed in the low-dose group (Bjørnevik and Maage 1993), and the differences observed may better be described as effects of iron loading. In acquaculture generally, feed levels of iron as high as 500 mg Fe/kg have been reported (Maage 1991). During the last few years, observations that such high dietary iron levels may adversly affect the immune system and lead to increased disease incidences and reduced growth rates (Rørvik et al. 1991) have made feed producers and fish farmers more conscious of iron levels in the feed. Excessive levels of dietary iron have also come under suspicion as a potentially adverse factor in human health, with indications that it can increase susceptibility to parasitic and bacterial infections (Keusch and Farthing 1986; Anonymous 1989). The mechanisms underlying these effects are poorly understood, however.

Tangerås (1983) has shown that iron loading in rats may result in the accumulation of iron in lysosomes (as hemosiderin), but not in mitochondria, where heme synthesis occurs. This observation is in agreement with the present finding that high-iron doses in salmon do not result in a general depression of heme proteins like the P450 isozymes. Rather, a differential regulation of different P450 subfamilies was seen, since as P450 1A1 went down, P450con (P450 3A) was stable. Bacon et al. (1986) observed a decrease in total P450 and aminopyrine demethylase activity, another P450-mediated process, in iron-overloaded rats. This study, however, did not include immunochemical studies of different isozymes.

Comparison of the correlation plots of the present results shows that EROD activity correlates very well with P450 1A1 levels in all control fish (Fig. 1A), in agreement with the general acceptance of P450 1A1 as the predominant EROD catalyst in fish (e.g., Goksøyr et al. 1991; Goksøyr and Förlin 1992). However, in BNF-treated fish, low correlation between EROD and P450 1A1 is seen whereas EROD and NADPH-cytochrome P450 reductase correlate well (Fig. 2). The finding indicates that after induction, electron transport through the reductase becomes rate limiting. Thus, after BNF treatment, reductase levels will determine EROD activity, independent of the presence of P450 1A1 protein. The turnover number for reductase (i.e., the ratio of reductase activity to total P450) is similar in all groups except the low-iron, BNF-treated group, which is much higher (368 compared with 176–214 nmol·min^{-1}·nmol P450^{-1}). However, when turnover numbers are expressed as the ratio between reductase and P450 1A1 levels (as ELISA Abs.), the picture is different. The low-, medium-, and high-iron diets produce isozyme-specific turnover numbers of 157, 200, and 341 nmol·min^{-1}·nmol P450 1A1^{-1}, respectively, in the control groups whereas the numbers are 151, 79, and 90 in the BNF groups (calculated from Table 2). These ratios are apparently not sufficient for maintaining a full pace of EROD activity with the available P450 1A1 protein.

In summary, the results show that dietary iron levels have selective regulatory effects on catalytic activities, specific isozyme pattern, and induction response of the cytochrome P450 system of Atlantic salmon. Selective regulation could involve translational control by iron-responsive elements (IREs) (Casey et al. 1988), but such elements have so far not been reported in any *CYP* gene. Obviously, further studies of the molecular mechanisms behind iron regulation of salmon P450s are needed.

The postulated involvement of P450 enzymes in regulation of important physiological processes such as homeostasis,

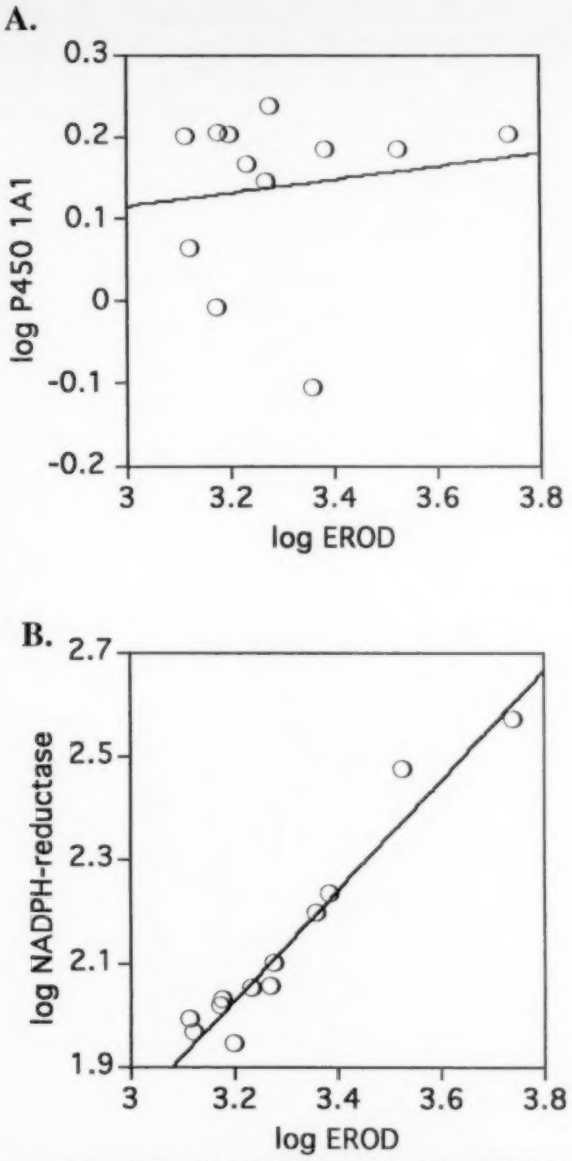

FIG. 2. Correlation plots of EROD activities with (A) P450 1A1 protein levels (measured as ELISA Abs.) and (B) NADPH-reductase activities in liver microsomes from Atlantic salmon given different dietary levels of iron (BNF group). Individual data from all feeding groups are log-transformed. Linear curve fits are represented by the lines (A) $f(x) = 0.081 \cdot x - 0.129$ ($R^2 = 0.021$) and (B) $f(x) = 1.063 \cdot x - 1.373$ ($R^2 = 0.944$).

growth, differentiation, and neuroendocrine functions (Nebert 1991) makes dietary effects like these important to establish. Although speculative at the moment, the effects observed may also be related to other observations of detrimental effects on disease resistance of high iron doses in the feed of Atlantic salmon (Rørvik et al. 1991). A more direct implication of the present findings are the antagonistic effects of iron on P450 1A1 induction, which need to be taken into consideration in field studies where different locations may result in different nutritional status of the fish, as well as different exposures to mixed (organic and inorganic) contaminant situations.

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Changes in the Diet of Demersal Fish due to Eutrophication-Induced Hypoxia in the Kattegat, Sweden

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Pihl, L. 1994. Changes in the diet of demersal fish due to eutrophication-induced hypoxia in the Kattegat, Sweden. Can. J. Fish. Aquat. Sci. 51: 321–336.

The diets of five dominant bottom-feeding fish species, Atlantic cod (*Gadus morhua*), whiting (*Merlangius merlangus*), plaice (*Pleuronectes platessa*), dab (*Limanda limanda*), and American plaice (*Hippoglossoides platessoides*), were analysed during spring and autumn between May 1984 and April 1988 in the south-east Kattegat. A general long-term change in the diet of bottom-feeding fish in the Kattegat has taken place since the beginning of the century and, for some of the species, the feeding pattern was different from that in adjacent areas. The observed dietary shift appeared to be due to changes in species composition of benthic macrofauna. Prey species favored by increased organic enrichment were dominant in the diet. Several infaunal species increased in dietary importance during recent hypoxia. In contrast, during hypoxia, a reduction of epibenthic crustaceans was observed in the fish diet. This study emphasizes the potential effects of organic enrichment and hypoxia (due to eutrophication) on trophic interactions in marine benthic communities. Repeated stress from hypoxia might favor small-sized prey species with a short life cycle, which would in turn favor small-sized fishes. Thus, altered food resources and the direct effects of hypoxia might result in shift in dominance among demersal fish species.

Le régime alimentaire de cinq espèces dominantes de poissons se nourrissant sur le fond, la morue franche (*Gadus morhua*), le merlan (*Merlangius merlangus*), la plie (*Pleuronectes platessa*), la limande (*Limanda limanda*) et la plie canadienne (*Hippoglossoides platessoides*) a été examiné au printemps et à l'automne, entre mai 1984 et avril 1988, dans le sud-est de Kattegat. Depuis le début du siècle, il s'est produit un changement général à long terme du régime alimentaire des poissons qui se nourrissent sur le fond à Kattegat, et pour certaines espèces, le profil d'alimentation différait de celui des régions voisines. Le changement observé semblait attribuable à des modifications dans la composition des espèces de la macrofaune benthique. Le régime alimentaire était dominé par des espèces-proies, favorisées par un enrichissement accru en matières organiques. L'importance alimentaire de plusieurs espèces appartenant à l'endofaune a augmenté au cours du phénomène récent d'hypoxie. Par contraste, en période d'hypoxie, on a observé une réduction des crustacés épibenthiques dans le régime alimentaire des poissons. Cette étude met l'accent sur les effets potentiels d'un enrichissement en matières organiques et d'une hypoxie attribuables à l'eutrophisation, sur les interactions trophiques dans les communautés benthiques marines. Un stress répété causé par une hypoxie pourrait favoriser les espèces de proie de petite taille à cycle biologique court, ce qui pourrait être favorable aux poissons de petite taille. Une altération des ressources alimentaires et les effets directs d'une hypoxie pourraient donc provoquer un changement de dominance chez les espèces de poissons démersaux.

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The Kattegat is a shallow (mean depth 23 m) transition area between the brackish Baltic Sea and the marine North Sea. It is a highly productive area with an important pelagic and demersal fishery. Total annual fish production has been estimated to be approximately 75 kg (wet weight)·ha^{-1}, which is higher than fish production estimates in both the North Sea (50 kg·ha^{-1}) and the Baltic (35 kg·ha^{-1}) (Rosenberg 1984). Over the last five decades, the Kattegat has received increasing amounts of nutrients, mainly nitrogen and phosphorus, and the southern part is now considered eutrophic (Rosenberg 1985). Increased primary production enhanced organic loading in the area, which in turn increased oxygen consumption in bottom water due to mineralization (Rydberg et al. 1990). Reduced oxygen concentrations in the bottom water have been quantified over a 3000-km^2 area in the southeast Kattegat from August to November each year since 1980 (Baden et al. 1990a). The severity and duration of hypoxia (O_2 < 3 mg·L^{-1}; 30% saturation) varies annually (3–10 wk). Most severe hypoxia with mean oxygen concentration below 1.4 mg·L^{-1} occurred for about 3 wk in September 1988, and concentrations below 3 mg·L^{-1} were measured for 10 wk during the same year.

Hypoxic events affect the benthic community structure adversely by killing several species (Rosenberg and Loo 1988; Baden et al. 1990a: Rosenberg et al. 1992). One of the most affected species, sedentary Norway lobster (*Nephrops norvegicus*), which is an important benthic predator and bioturbator in the Kattegat, has been virtually eliminated from a 3000-km^2 area since 1988 (Baden et al. 1990b). Furthermore, total biomass of demersal fish was reduced during hypoxia by 50–80% (Pihl 1989; Baden et al. 1990a) During severe hypoxia in September 1988, demersal fish

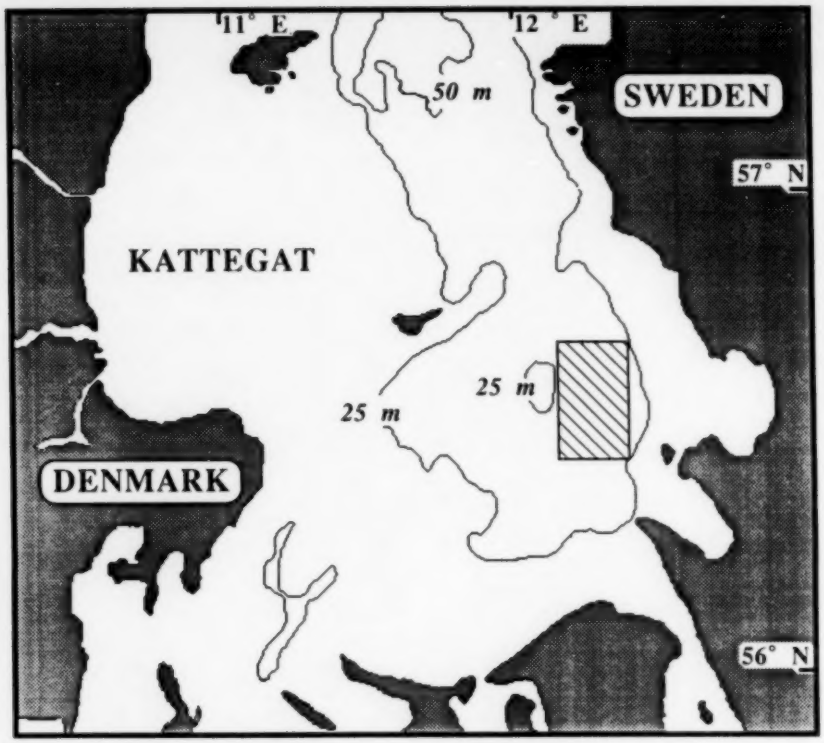

FIG. 1. Map of the Kattegat showing the study area (hatched rectangle) where trawl samples were collected at six stations between 25 and 40 m depth. Depth curves 25 and 50 m are indicated.

biomass was only 2% of that during normoxic conditions. Tolerance to low oxygen varies in demersal fish species, thereby dramatically changing species composition during hypoxia (Pihl 1989). Similar effects of hypoxia on benthic infauna and demersal fish were reported for some years during the 1980's in adjacent Kiel Bay and the North Sea (Dyer et al. 1983; Weigelt and Rumohr 1986).

In the Kattegat, interactions between demersal fishes and their infaunal prey items are of major importance, as most of the energy flow is through the benthic system (Pearson and Rosenberg 1992). This investigation documents long-term changes in the diet of five fish species composing over 80% of the demersal fish biomass in the Kattegat (Pihl 1989) and evaluates interactions between bottom water hypoxia and changes in diet. Dietary composition of conspecifics from adjacent unaffected areas was used as a basis for comparison. The diets of Atlantic cod (*Gadus morhua*), whiting (*Merlangius merlangus*), plaice (*Pleuronectes platessa*), dab (*Limanda limanda*), and American plaice (*Hippoglossoides platessoides*) were analysed during spring and autumn on 10 occasions between May 1984 and April 1988 in the southeast Kattegat.

Study Area

This study was carried out at 25–40 m depth in the southeast Kattegat (56°30′N; 12°20′E) (Fig. 1), which is characterized by strong vertical salinity stratification: a low-salinity (15–25‰) Baltic surface current overlys high-salinity (32–34‰) bottom water entering from the Skagerrak and results in a halocine normally situated 15–20 m deep. When a thermocline also develops during summer, stratification is even stronger and mixing between the two water bodies is further reduced. Oxygen consumption during autumn is high (0.1–0.7 $mg \cdot L^{-1} \cdot d^{-1}$) in the bottom water (Rydberg et al. 1990) resulting in hypoxia in most years during the 1980's (Baden et al. 1990a). The tidal amplitude in the area is about 0.1 m (Svansson 1975). The sediment structure in the area varies from sandy silt (shallow parts) to silty mud (deeper parts), and organic matter in the sediment (measured as ignition loss) ranges from 5 to 15% (Rosenberg and Loo 1988).

Materials and Methods

Fish were sampled with a Glommen lobster trawl (ground rope 86 m, head rope 76 m, sweep 55 m, and otter-board 2.2 m) with a mesh size of 60 mm in the cod end. Eleven trawl surveys were carried out in spring (April–May) and autumn (September–December) during 1984–88 (Fig. 2). On each occasion, fish were collected from five to six daytime trawl samples except during the last sampling occasion in September 1988 when too few fish were caught in the area for stomach analysis. Fish standard length was determined on the ship and species were separated into the following small, medium, and large size-classes: Atlantic cod (19–24, 25–49, and 50–74 cm), plaice (20–29 and 30–39 cm), and whiting, dab, and American plaice (15–19, 20–24, and

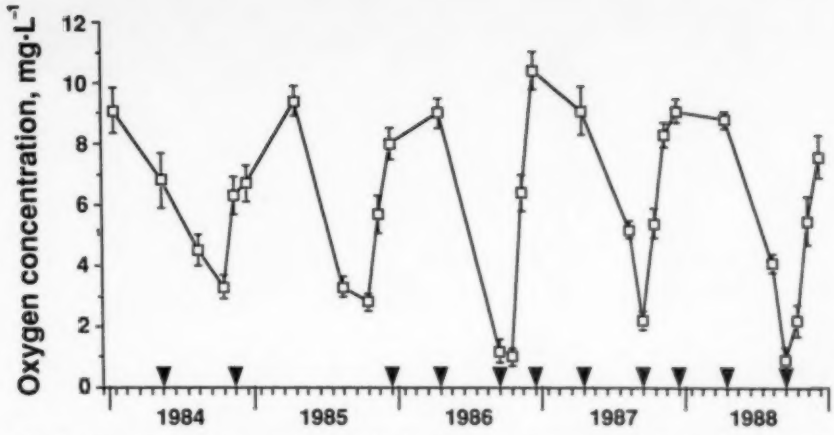

FIG. 2. Mean (and SD) oxygen concentration in the bottom water in the study area in the southeast Kattegat from 1984 to 1988. Measurements where conducted at five to six stations (<0.5 m above the sediment surface) on each sampling occasion. Dates for demersal fish sampling are indicated by arrows.

25–29 cm). The two size-classes of plaice were later pooled into a 20–39 cm size-class because only small dietary differences were found between the groups. If available, stomachs from 10 individuals of each size-class per species were taken from each trawl sample. If fewer than 25 individuals per size-class (five individuals from each trawl sample) were collected on any sample date, they were not included in the analysis. Fish with empty stomachs were few (5–10%) and were excluded. The investigation was based on analysis of 1250, 1580, 1080, 1440, and 1480 stomachs from Atlantic cod, whiting, plaice, dab, and American plaice, respectively. Stomachs were excised and preserved in 70% ethanol within 1 h after catch for further analysis in the laboratory where all food items were identified to the lowest taxon possible and weighed (ethanol wet weight) within 2 wk to the nearest 0.1 mg. The mean weight of each food item (species or group) was calculated for the different size-classes in each trawl sample, and for each sampling occasion the mean percentage of the total biomass of all food items were determined.

Bottom water dissolved oxygen was measured at all trawl sample sites and averaged over the study area for each sampling date. Additional measurements made during other studies in the area were also used to quantify seasonal variations in oxygen. Measurements were made within 0.5 m of the sediment surface using an air and Winkler calibrated SC-28YSI-oxygen meter. The vertical distribution of oxygen was measured with a probe at every metre in the water column on all sampling occasions. On 5 of 10 sampling dates, Winkler titrations were made concurrently on samples taken with a water bottle for comparison of bottom water oxygen concentration. Differences between in situ measurements made with the two methods generally did not exceed about 0.1 $mg \cdot L^{-1}$.

Results

Oxygen Concentration

Mean dissolved oxygen concentration of the bottom water for 1984–88 exhibited similar seasonal variability (Fig. 2): oxygen concentrations were high during winter, decreased from spring to autumn, and were always lowest in September and October, with levels gradually increasing to normal in December during water column destratification. Hypoxic conditions (O_2 concentration <3.0 $mg \cdot L^{-1}$; 30% saturation) occurred for 4, 9, 5, and 10 wk during 1985, 1986, 1987, and 1988, respectively, whereas in autumn 1984, oxygen deficiency was less severe. The lowest mean oxygen concentration (O_2 < 2.0 $mg \cdot L^{-1}$) in the bottom water was recorded during September 1986, 1987, and 1988. Hypoxia extended about 10 m above the seabed in the autumn 1988 compared with about 1 m in previous years.

Overall Diet

Overall dietary components of the five fish species studied were separated into 101 different food items from five major taxa (Table 1). Atlantic cod and whiting had similar diets consisting primarily of crustaceans and fish and secondarily of polychaetes. *Crangon crangon, C. almani,* cumaceans, and *Meganyctiphanes norvegica* were the dominant crustaceans eaten by both species, but cod also consumed crabs (*Liocarcinus depurator* and *Pagurus bernhardus*) and shrimp (*Pandalus borelis*). Dominant fish species taken by cod and whiting included Atlantic herring (*Clupea harengus*) and sprat (*Sprattus sprattus*), but dragonet (*Callionymus lyra*), spotted dragonet (*C. maculatus*), whiting, and snake blanny (*Lumpenus lampretaeformis*) were also abundant in cod stomachs. Whiting also consumed dragonet and lesser sand-eel (*Ammodytes lancea*). *Aphrodite aculeata* was by weight the most important polychaete for Atlantic cod. Whiting generally consumed fewer polychaetes, but *Nepthys* sp. were eaten to some extent. Echinoderms and molluscs made up a small proportion of the diet in these species.

Polychaetes were the main food of plaice and were dominated by *Polyphysia crassa*, which contributed 35% of the total diet in this species. Other polychaetes in the diet included *Nephtys* sp. and *Scalibregmidae*. Echinoderms (principally *Amphiura filiformis*) and molluscs (*Abra alba* and *A. nitida*) were the next most important dietary groups for

TABLE 1. Mean overall diet for cod, whiting, plaice, dab, and American plaice during spring and autumn from 1984 to 1988 in the study area (25–40 m depth) in the southeast Kattegat. Diet is separated into 101 food items and given as percentage of total biomass of food consumption, an asterisk indicating <0.1% of total biomass.

Food items (% of total biomass)	Cod	Whiting	Plaice	Dab	American Plaice
Crustacea	39.7	29.7	2.5	6.1	14.8
Amphipoda	*	0.1		0.5	*
Balanus improvisus (Darwin)	*		*		
Carcinus maenas (L.)	0.5			0.3	*
Corophium sp.				*	
Crangon almani (Kinahan)	10.7	10.0			0.9
Crangon crangon (L.)	1.4	0.4			1.3
Crangon sp.	6.2	3.8		0.3	4.6
Cumacea	2.3	4.6	0.0	0.8	0.3
Diastylis rathkei (Krøyer)	0.3	0.4	0.2	0.4	0.2
Eudorella emarginata (Krøyer)	*	0.5	0.4	*	*
Galathea sp.	*	0.1			
Idothea sp.	*	*			
Leucon nasica (Krøyer)	*	*			
Liocarcinus depurator (L.)	3.6	*		0.2	0.3
Meganyctiphanes norvegica (M. Sars)	3.8	6.1		3.2	6.6
Mysidae	1.4	0.7	*		0.3
Nephrops norvegicus (L.)	1.3				
Ostracoda			*	0.7	*
Pagurus bernhardus (L.)	4.3	0.4	1.9	0.5	0.2
Pandalus borealis (Krøyer)	2.4	0.3			
Philomedes globosus (Lilljeborg)				*	
Tanaidacea	*				
Echinodermata	1.3	0.1	17.0	70.4	62.6
Amphiura chiajei (Forbes)	*		0.1	2.6	4.3
Amphiura filiformis (O.F. Müller)	0.8	*	5.9	21.5	15.4
Amphiura sp.	*		8.2	41.5	26.2
Echinocardium cordatum (Pennant)				*	
Echinus esculentus (L.)	0.1			0.1	0.3
Ophiura albida (Forbes)	0.1	*	*	0.4	5.4
Ophiura sarsi (Lütken)					*
Ophiura sp.	*		*	4.1	9.2
Ophiura texturata (Lamarck)				*	
Pseudocucumaria sp.	0.2		0.5		
Mollusca	1.1	0.0	16.5	4.8	4.6
Abra alba (W. Wood)			1.2		
Abra nitida (Müller)	*		8.4	0.3	0.2
Abra sp.	*	*	4.8	3.6	1.8
Acera sp.				*	
Arctica islandica (L.)	0.2		2.1		
Astarte montagui (Dillwyn)	*		*		
Buccinum sp.			0.1		
Bivalvia	0.8		1.2	0.1	0.3
Cephalopoda	0.3				
Cardium edule (L.)	*		*	*	*
Corbula gibba (Olivi)			*	*	*
Cultellus pellucidus (Pennant)				*	
Modiolus modiolus (L.)				*	
Mysella bidentata (Montagu)				*	
Mya sp.	*		0.2	*	*
Mytilus edulis (L.)	*				
Nucula tenuis (Montagu)			0.8	*	*
Nuculana pernula (Müller)			*	*	2.3
Philine sp.			0.1	0.7	*
Retusa obtusa (Montagu)				*	
Retusa truncatula (Bruguière)				*	
Spisula sp.			*		

TABLE 1. (*Concluded*)

Food items (% of total biomass)	Cod	Whiting	Plaice	Dab	American Plaice
Tellina tenuis (de Costa)				*	
Turitella communis (Risso)			*		
Polychaeta	12.7	7.3	58.2	11.2	3.2
Ampharetidae			0.1	*	*
Anaitides sp.	*			*	
Aphrodite aculeata (L.)	6.9	*	*	0.1	
Brada villosa (Rathke)	0.8		0.4	0.3	*
Diplocirrus sp.		*	*	0.1	*
Eteone sp.	*		*		
Flabelligeride	0.1		0.4	0.2	*
Glycera sp.	*	0.1	0.9	0.7	*
Heteromastus filiformis (Claparède)		0.2			*
Maldane sarsi (Malmgren)	*	*	*	1.1	*
Myriochele sp.			*	*	*
Nephtys sp.	0.3	1.8	3.9	1.9	1.4
Nereis sp.	0.2	0.5	*	0.5	
Notomastus sp.			0.3		
Ophelidae				0.2	
Pectinaria sp.	0.7	0.2	0.5	1.4	0.1
Pherusa plumosa (O.F. Müller)			0.4		
Phole sp.				*	*
Phyllodocidae	0.3	0.1		*	
Phyllodoce groenlandica (Örsted)				*	
Polyphysia crassa (Örsted)		0.4	34.8	1.8	0.2
Rhodine sp.					
Sabellidae				*	
Sagarlidae		0.1			
Scalibregma inflatum (Rathke)	*	0.2	0.2	1.2	0.2
Scalibregmidae	*		4.7	*	
Sosane gracilis (Malmgren)					*
Sphaerodrum sp.			*	*	
Terebellidae	0.2	*	0.2	*	0.3
Trochochaeta multisetosa (Örsted)		*		*	
Pisces	42.6	59.5	0.0	4.3	14.0
Ammodytes lancea (Cuvier)		1.8			0.1
Callionymus lyra (L.)	2.8	5.9			
Callionymus maculatus (Rafinesque-Schmaltz)	2.5				*
Clupea harengus (L.)	6.8	28.0		3.2	5.6
Clupeidae	12.5	8.8			
Clupeidae, larvae				0.1	5.7
Gobidae	0.2	0.8			2.4
Hippoglossoides platessoides (Fabricius)	1.4				
Merlangius merlangus (L.)	2.6	0.3			0.2
Sprattus sprattus (L.)	6.0	10.0			
Lumpenus lampretaeformis (Walbaum)	2.5				
Others	2.5	3.0	5.8	3.4	0.9
Burrowing anthozoan	*			*	
Nematoda	*				*
Nemertini				0.1	
Oligochaeta	*		*		
Polyclada	0.3			1.7	*
Pycnogonum littorale (Strøm)	*	2.0			0.8
Priapulus caudatus (Shipley)	2.0		5.8	1.6	0.1

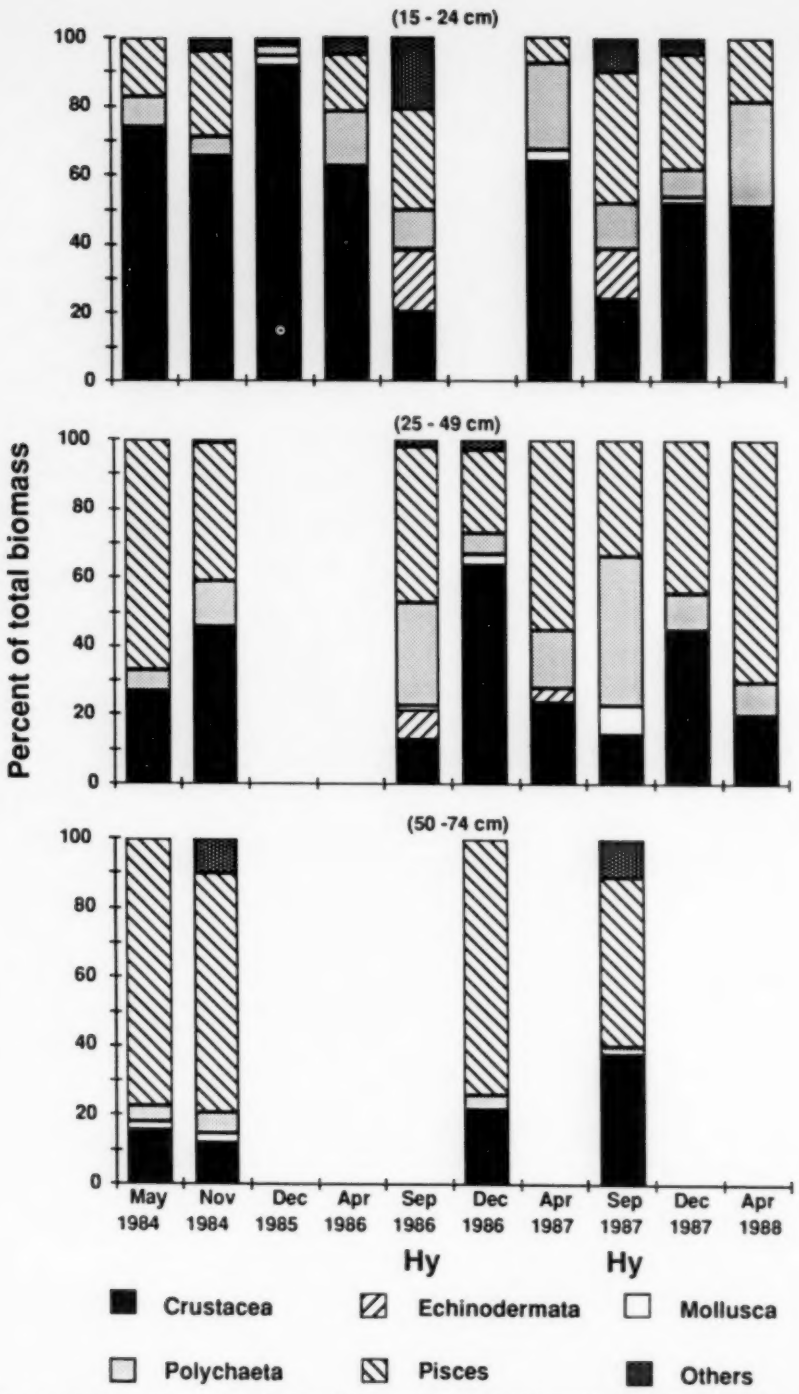

FIG. 3. Percentage of total biomass for Crustacea, Echinodermata, Mollusca, Polychaeta, Pisces and other prey items in the diet of three length-classes (15–24, 25–49, and 50–74 cm) of Atlantic cod (*Gadus morhua*) in the southeast Kattegat during 10 sampling occasions from 1984 to 1988. Hy indicates a period with hypoxia.

plaice. The dominant crustacean taken by plaice was the hermit crab (*Pagurus bernhardus*).

Dab and American plaice both fed selectively on echinoderms dominated by amphiuroids and ophiuroids, which composed 70 and 63% of the diet in these fish species, respectively. Amphiuroids were often difficult to identify

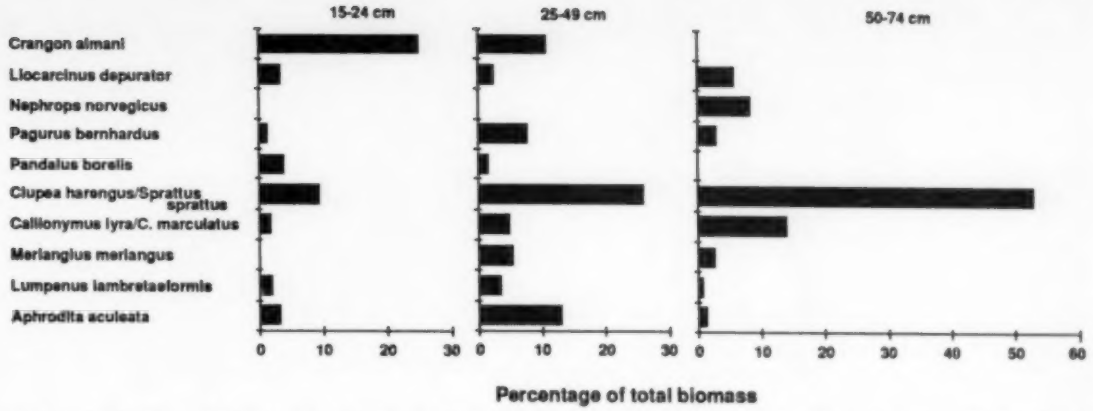

FIG. 4. Percentage of total biomass for the 10 most important prey items in the diet of three length-classes (15–24, 25–49, and 50–74 cm) of all Atlantic cod sampled in the southeast Kattegat from 1984 to 1988.

TABLE 2. Importance of some dominant prey items in the diet of cod, plaice, dab, and American plaice during normoxia (6.4–10.3 $mg \cdot L^{-1}$) (November 1984, December 1985, December 1986, and December 1987) and hypoxia (<3 $mg \cdot L^{-1}$) (September 1986 and September 1987) in the southeast Kattegat. Percentage (range and mean) of total biomass in the diet is given for each prey item.

Prey item	Predator (length, cm)	Normoxia (% of total biomass) range (mean)	Hypoxia (% of total biomass) range (mean)
Crustacea	Cod (19–24)	54–91 (70)	22–24 (23)
	Cod (25–49)	45–63 (51)	13–15 (14)
	American plaice (20–24)	6–88 (34)	0.7–1.4 (1)
Pagurus bernhardus	Plaice (20–39)	0	0–23 (11.5)
Amphiura spp.	Cod (19–24)	0	12–17 (14.5)
	Cod (25–49)	0–0.6 (0.3)	0.1–6 (3)
	Plaice (20–39)	3–22 (13)	22–26 (24)
	Dab (15–19)	72–76 (74)	81–84 (82.5)
	Dab (20–24)	38–65 (47)	66–88 (77)
	American plaice (15–19)	23–48 (36)	79–91 (85)
	American plaice (20–24)	6–75 (31)	83–90 (86.5)
Polychaeta	Plaice (20–39)	61–76 (68)	19–46 (32.5)
Aphrodite aculeata	Cod (25–49)	3–8 (5)	27–43 (35)

to species because arm fragments of these animals dominated in the stomachs. However, when they could be identified, *Amphiura filiformis* contributed 90 and 80% of amphiuroid numerical abundance in dab and American plaice stomachs, respectively. *Ophiura albida* made up about 98% of the ophiuroid biomass in the diet of both fishes. Polychaetes (*Maldane sarsi, Nephtys* sp., *Pectinaria* sp., *Polyphysia crassa*, and *Scalibregma inflatum*) were of secondary importance for dab whereas crustaceans (*Crangon* spp. and *Meganyctiphanes norvegica*) and fish (Atlantic herring and gobiids) were secondary food items in American plaice.

Within the "Others" group (Table 1), *Priapulus caudatus* was an important food for Atlantic cod, plaice, and dab whereas *Polycladida* and *Pycnogonum littorale* were eaten by whiting and dab.

Fish Size and Seasonal Dietary Relationships

Atlantic cod (Gadus morhua)

Small (15–24 cm) — Crustaceans contributed over 50% of the food biomass eaten on all sampling occasions except during the hypoxia in September 1986 and 1987 when they made up only about 20% of the biomass consumed (Fig. 3; Table 2). *Crangon almani, Liocarcinus depurator, Pandalus borealis*, and *Pagurus bernhardus* were the dominant crustaceans taken by small cod (Fig. 4). Fish and polychaetes were the next most important food for young cod, with fish contributing 29, 37, and 33% of consumed biomass during hypoxia in September 1986 and 1987 as well as in December 1987, respectively. Both demersal and pelagic fish were eaten and no species were particularly selected. Polychaetes

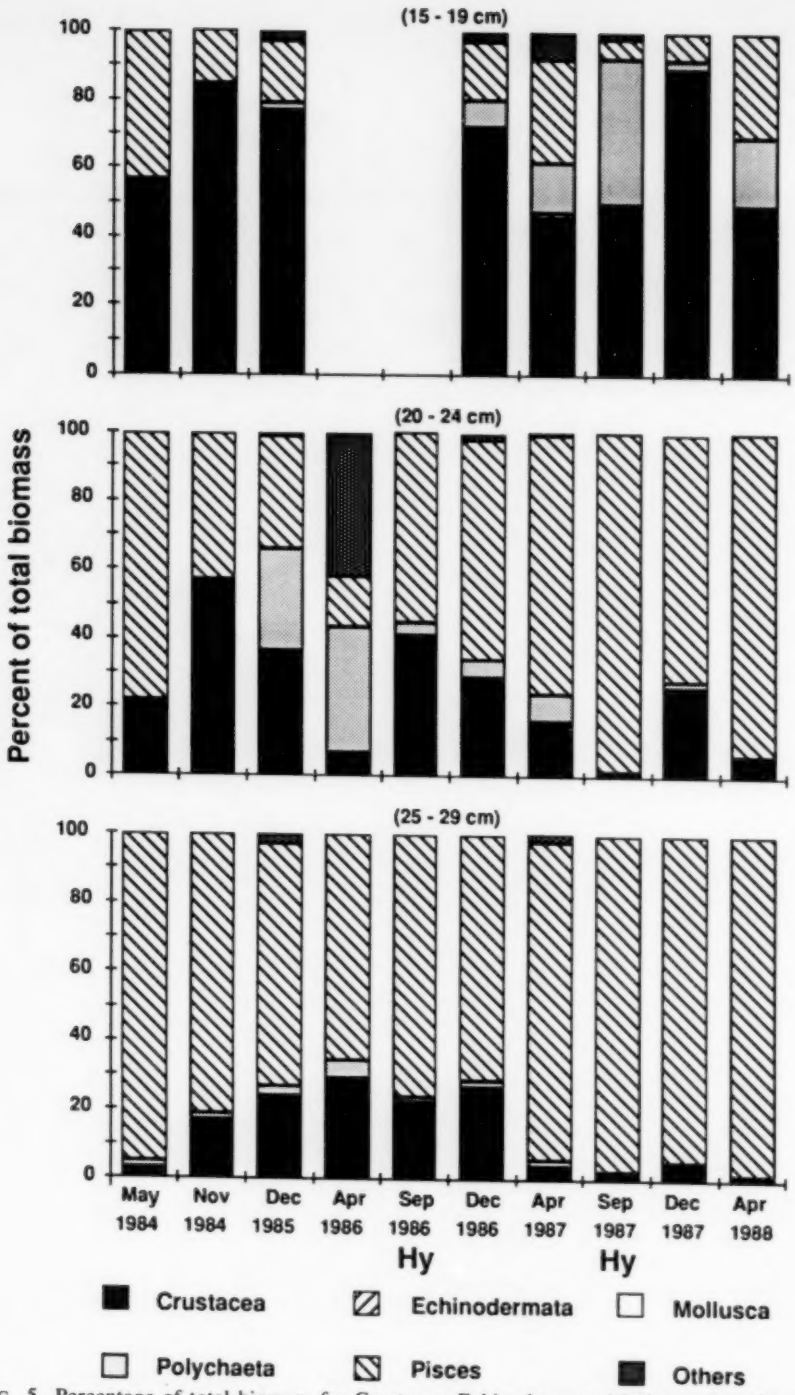

FIG. 5. Percentage of total biomass for Crustacea, Echinodermata, Mollusca, Polychaeta, Pisces, and other prey items in the diet of three length-classes (15–19, 20–24, and 25–29 cm) of whiting (*Merlangius merlangus*) in the southeast Kattegat during 10 sampling occasions from 1984 to 1988. Hy indicates a period with hypoxia.

were mainly eaten in spring when they contributed 9–30% of the biomass consumed. *Aphrodite aculeata, Brada villosa,* and *Pectinaria* sp. were the most common polychaetes in stomachs of small cod and, of other species, *Priapulus caudatus* was dominant. Echinoderm and mollusc biomass was generally <1% of total in the stomachs of small cod,

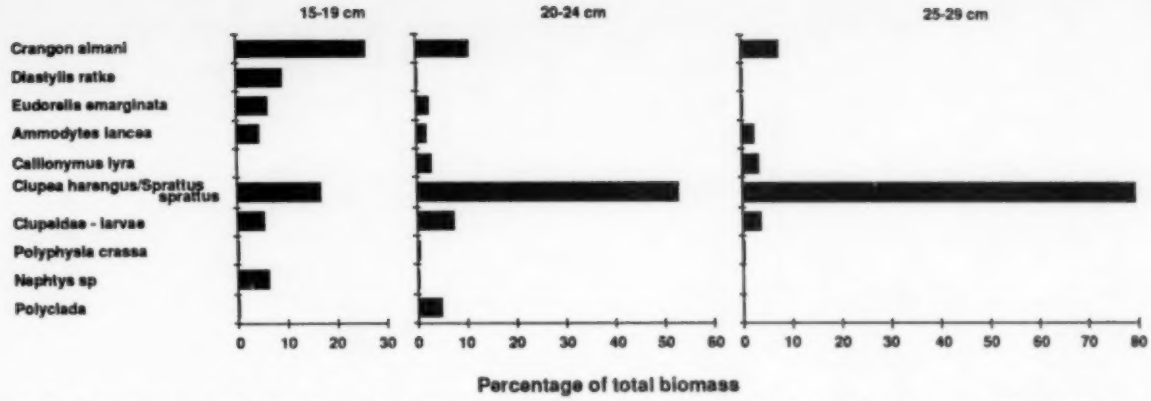

FIG. 6. Percentage of total biomass for the 10 most important prey items in the diet of three length-classes (15–19, 20–24, and 25–29 cm) of all whiting sampled in the southeast Kattegat from 1984 to 1988.

but the brittle star *Amphiura filiformis* was eaten during the periods of hypoxia in September 1986 and 1987, contributing 17 and 12% of total food consumed, respectively (Table 2).

Medium (25–49 cm) — On average, fish contributed 50% and crustaceans 30% of the food biomass in this size-class (Fig. 3). No clear seasonal variation in fish consumption could be seen. Similar to small cod, the lowest percentage contribution from crustaceans (about 15%) occurred during hypoxia in September 1986 and 1987 (Table 2). Polychaetes generally contributed up to 10% of the biomass consumed, except during hypoxia in September 1986 and 1987 when *Aphrodite aculeata* alone made up 27 and 42% of the total food consumed, respectively. Echinoderms and molluscs occurred infrequently in the stomachs, except for the brittle star *Amphiura filiformis* which was eaten in September 1986.

Clupea harengus and *Sprattus sprattus* were the principal pelagic fish consumed and contributed to 26% of gut contents overall (Fig. 4). Demersal fish made up 18% of all food consumed. As in small cod, the same dominant crustacean prey were also consumed by medium-sized cod, and *Pagurus bernhardus* was also eaten by this size-class. *Aphrodite aculeata* made up 83% of the biomass of all polychaetes eaten.

Large (50–74 cm) — Large cod were found only on four occasions in numbers high enough for dietary analysis; therefore, seasonal trends could not be evaluated (Fig. 3). Pelagic fish (*Clupea harengus* and *Sprattus sprattus*) made up 55% of the diet whereas demersal fish (*Callionymus lyra* and *Merlangius merlangus*) were infrequently eaten (Fig. 4). The dominant crustaceans in large cod were *Nephrops norvegicus, Liocarcinus depurator,* and *Pagurus bernhardus.* Few molluscs and polychaetes were consumed and echinoderms were absent in the diet.

Whiting (Merlangius merlangus)

Small (15–19 cm) — Crustaceans (principally *Crangon almani* and cumaceans) were the dominant food on all occasions (Fig. 5 and 6). Pelagic fish (*Clupea harengus, Sprattus sprattus,* and *Ammodytes lancea*) were ranked second in gut contents. Polychaetes including *Aphrodite aculeata, Nephtys* sp., and *Pectinaria* sp. were eaten infrequently whereas echinoderms and molluscs were rare in the stomachs.

Medium (20–24 cm) — *Clupea harengus, Sprattus sprattus,* and *Crangon almani* were the dominant prey (Fig. 6), but on two sampling occasions (December 1985 and April 1986), polychaetes (*Nereis* sp., *Polyphysia crassa,* and *Scalibregma inflatum*) were also abundant (Fig. 5).

Large (25–29 cm) — Large whiting consumed pelagic fish (dominated by *Clupea harengus* and *Sprattus sprattus*) almost exclusively (Fig. 5 and 6). On five sampling occasions, crustaceans (*Crangon almani* and cumaceans) were also eaten. The proportion of crustaceans in the diet was higher ($p = 0.008$, Mann–Whitney *U*-test) during the period 1985–86 compared with 1987–88. Other food items in stomachs were rare.

Plaice (Pleuronectes platessa)

20–39 cm — Polychaetes were the dominant food items for plaice on 7 of 10 sampling occasions, contributing 60–80% of the biomass consumed (Fig. 7). *Polyphysia crassa* was the most commonly eaten species, but other polychaetes such as *Nephtys* sp. and *Scalibregma inflatum* were also abundant (Fig. 8). In September 1986 and 1987 and in April 1988, echinoderms and molluscs together made up more than 50% of the gut contents. *Abra nitida* and *Amphiura filiformis* were the second most important food items for plaice, contributing about 80% of all molluscs and echinoderms taken, respectively. Another important species was *Priapulus caudatus.* Crustaceans were of little importance in the diet of plaice, except during hypoxia in September 1987 when soft parts of *Pagurus bernhardus* were found in the stomachs (Table 2). Fish were not eaten by plaice.

Dab (Limanda limanda)

Small (15–19 cm) — Echinoderms were the dominant food on all sampling occasions, especially during hypoxia in September 1986 and 1987 and in April 1988 (Fig. 9; Table 2). On average, the brittle star *Amphiura filiformis* made up 60% of all food taken (Fig. 10), but during some occasions, it contributed over 80%. *Amphiura chiajei* and *Ophiura albida* were the next common species found whereas other species were rare. Polychaetes were the next most important food and were taken mainly in spring. Crustaceans were found in small amounts in the stomachs on all sampling occasions. Fish, molluscs, and other groups of food were of little importance, except on one occasion when *Polycladida* was abundant.

Medium (20–24 cm) — Echinoderms also dominated the diet of this size-class but polychaetes and molluscs were

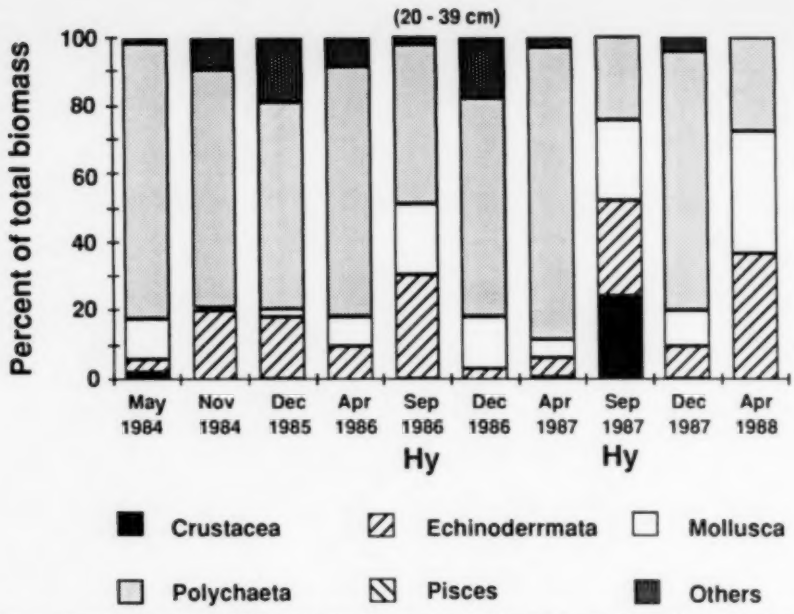

FIG. 7. Percentage of total biomass for Crustacea, Echinodermata, Mollusca, Polychaeta, Pisces, and other prey items in the diet of one length-class (20–39 cm) of plaice (*Pleuronectes platessa*) in the southeast Kattegat during 10 sampling occasions from 1984 to 1988. Hy indicates a period with hypoxia.

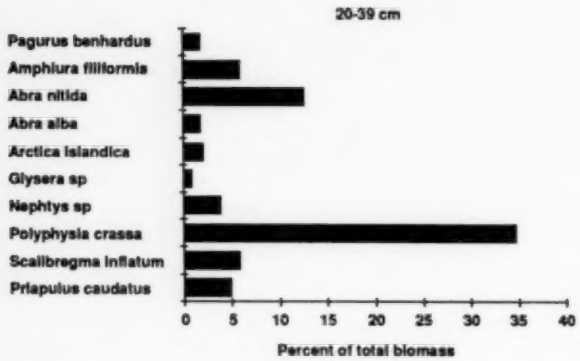

FIG. 8. Percentage of total biomass for the 10 most important prey items in the diet of one length-class (20–39 cm) of all plaice sampled in the southeast Kattegat from 1984 to 1988.

more important than in small dab (Fig. 9). Echinoderms were especially eaten in autumn, and polychaetes were mainly taken in spring. *Amphiura filiformis* and *A. chiajei* were the dominant species followed by *Abra nitida, Ophiura albida, Polyphysia crassa,* and *Priapulus caudatus* (Fig. 10). *Abra nitida* was taken in large amounts after the hypoxia in December 1986. Crustaceans were of little importance, except in November 1985 when *Meganyctiphanes norvegica* made up 50% of the food.

Large (25–29 cm) — These fish consumed mainly echinoderms in the autumn, but in spring, fish and polychaetes were also important (Fig. 9). *Amphiura filiformis, A. chiajei,* and clupeid fish were the dominant food items for large dab (Fig. 10).

American plaice (Hippoglossoides platessoides)

Small (15–19 cm) — Crustaceans, echinoderms, and fish were the primary food items for these fish (Fig. 11). In 1984 the crustaceans *Crangon* spp. and *Meganyctiphanes norvegica* were the dominant items, but in the other years, echinoderms (*Amphiura filiformis, A. chiajei,* and *Ophiura albida*) were eaten. During hypoxia in September 1986 and 1987, echinoderms (mainly *Amphiura filiformis*) contributed over 95% of the food taken by small American plaice (Table 2). Clupeid larvae and gobiids were eaten in April 1986 and 1987 and in December 1987. Molluscs and polychaetes were rare in the stomachs, but were found in small amounts in April 1986 and 1987. Brittle stars, dominated by *Amphiura filiformis, A. chiajei,* and *Ophiura albida,* made up the bulk of the diet, and other species like *Crangon crangon, C. almani,* Gobiidae, Clupeidae, and *Abra nitida* together contributed 17% of all food consumed (Fig. 12).

Medium (20–24 cm) — Echinoderms were the overall dominant food taken by this size-class, but crustaceans and fish also made up a large part of the food on some occasions (Fig. 11). The dominant food items were the brittle stars *Amphiura filiformis, A. chiajei,* and *Ophiura albida,* followed by the shrimps *Crangon crangon* and *C. almani* and clupeid larvae (Fig. 12). During the periods of hypoxia in September 1986 and 1987, echinoderms (mostly *Amphiura filiformis*) made up 95% of all food taken (Table 2). Molluscs were consumed in May 1984 and polychaetes in December 1987. These groups were rarely eaten on other sampling dates.

Large (25–29 cm) — Echinoderms were the most important food on all sampling occasions, except in April 1987 when fish and molluscs were abundant (Fig. 11). *Amphiura filiformis, A. chiajei,* and *Ophiura albida* were the most consumed species overall (Fig. 12). Similar to smaller

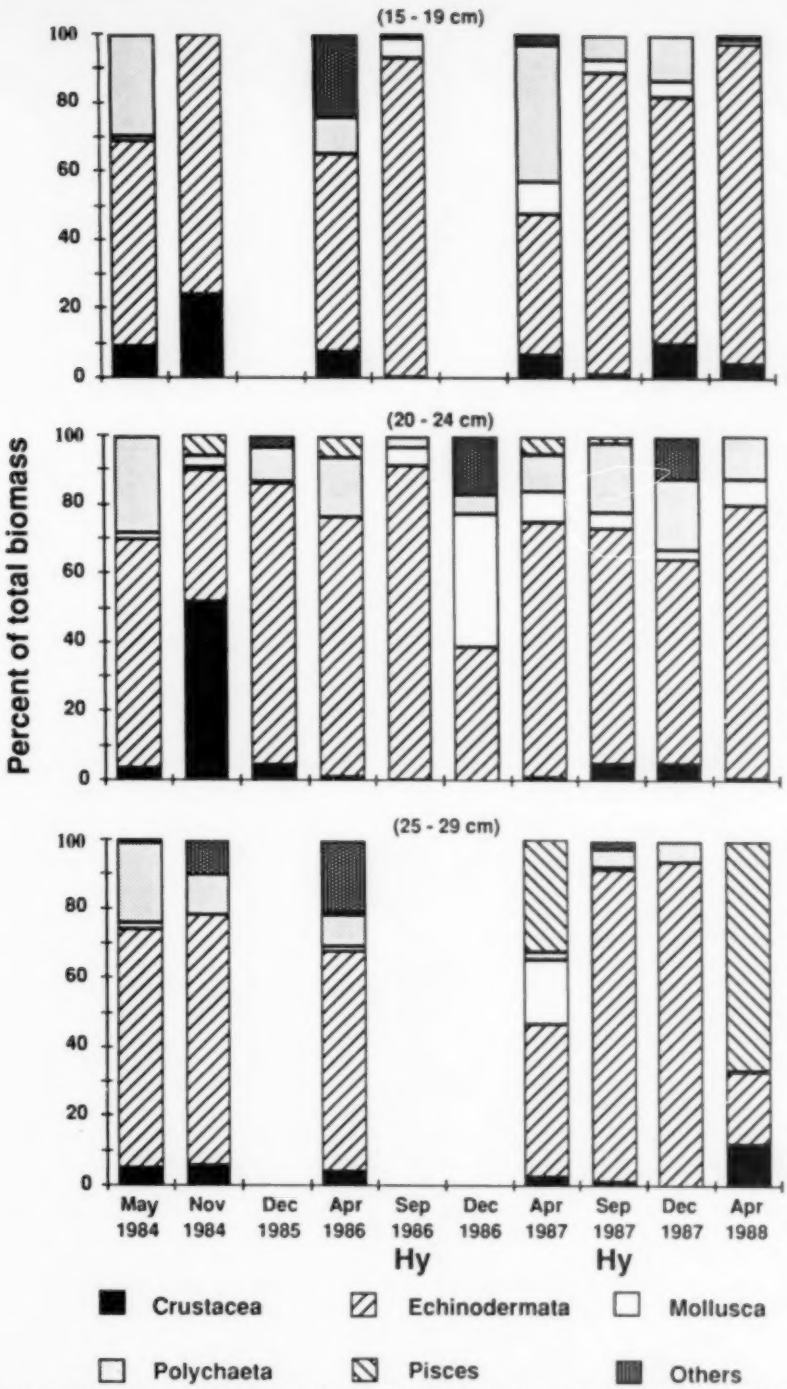

FIG. 9. Percentage of total biomass for Crustacea, Echinodermata, Mollusca, Polychaeta, Pisces, and other prey items in the diet of three length-classes (15–19, 20–24, and 25–29 cm) of dab (*Limanda limanda*) in the southeast Kattegat during 10 sampling occasions from 1984 to 1988. Hy indicates a period with hypoxia.

American plaice, echinoderms were eaten during the two periods of hypoxia in September 1986 and 1987. Crustaceans were taken in small amounts on most occasions whereas polychaetes were eaten in spring 1984 and 1986. Clupeidae larvae were consumed in April 1987 when *Abra nitida* also was eaten.

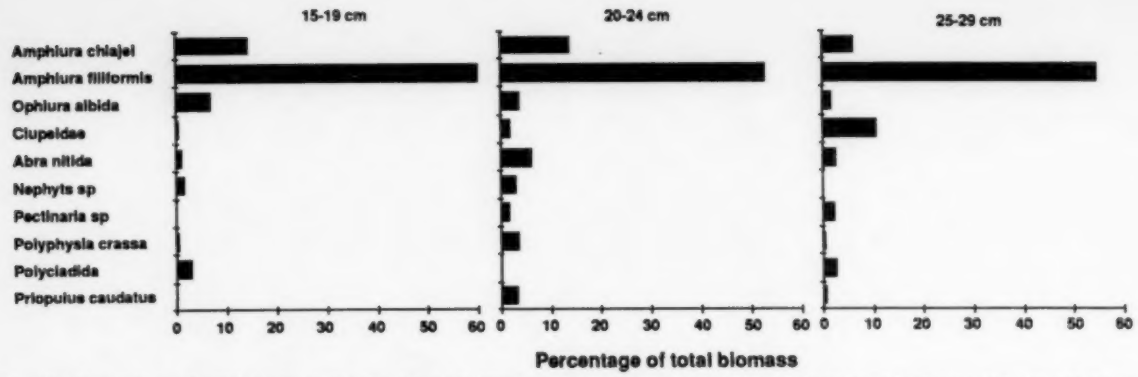

FIG. 10. Percentage of total biomass for the 10 most important prey items in the diet of three length-classes (15–19, 20–24, and 25–29 cm) of all dab sampled in the southeast Kattegat from 1984 to 1988.

Discussion

General Patterns and Long-term Changes in Diet

The diet of the five demersal predatory fish in the southeast Kattegat was highly diverse and comprised over 100 taxa including infaunal and epibenthic invertebrates as well as demersal and pelagic fish. Over 80% of the total biomass of demersal fish sampled in the area was composed of the species studied (Pihl 1989) and, together with Norway lobster (*Nephrops norvegicus*), constitute the major large predators in the southeast Kattegat (Baden et al. 1990b).

In general, the diet of Atlantic cod and whiting was similar to that reported in previous studies from the Kattegat (Blegvad 1916; Bagge 1978; Börje et al. 1987) and adjacent areas such as the Skagerrak (Mattson 1990; Hop et al. 1992), Kiel Bay (Arntz 1980; Arntz and Finger 1981), and the North Sea (Jones 1954; Daan 1973; Casey et al. 1984; Cramer and Daan 1986; Hislop et al. 1991). Crustaceans decreased and fish increased in importance with increasing size for both species. Similar dietary patterns for cod and whiting were reported from Kiel Bay and the North Sea (Jones 1954; Arntz 1974, 1980; Casey et al. 1984), indicating that gadoid diets are similar throughout northern European waters. The major prey of cod and whiting have not changed historically in the Kattegat. One exception, however, was that the dietary ratio of pelagic to demersal fish in this study was at least two times higher when compared with either investigations in the Kattegat (Blegvad 1916; Bagge 1978) and Kiel Bay and the North Sea (Arntz 1980; Casey et al. 1984). This could be due to increased dominance of pelagic species in the Kattegat during the last decade.

Plaice in the study area fed primarily on polychaetes (60% of biomass consumed) similar to plaice from the North Sea (50% of biomass consumed) (De Clerch and Buseyne 1989). Investigations from the Öresund also showed polychaetes to be dominant food items for plaice (45% of biomass consumed), together with molluscs (50% of biomass consumed) (Degel and Gislason 1988). *Polyphysia crassa* was of particular importance in the Kattegat and made up 35% of all food consumed, but was not among selected prey species in the North Sea or in the Öresund. Investigations from the beginning of the century in the Kattegatt showed that polychaetes and molluscs contributed 60 and 35%, respectively, of the food taken at depths below the halocline (Blegvad 1930).

Bagge (1978) reported that at depths below 20 m in the east Kattegat from 1950 to 1964, plaice consumed polychaetes (mainly *Nephtys* sp. and *Polyphysia crassa*) followed by molluscs (*Tellina tenuis* and *Nucula tenuis*). Echinoderms were found in low numbers in plaice stomachs analysed by Bagge (1978) but not those analysed by Blegvad (1930). These results suggest a dietary shift in plaice caught below the halocline from molluscs to echinoderms over the last five decades. Polychaetes were still the dominant faunal group eaten by plaice, although *P. crassa*, the main food during the 1980's, was of little importance (<1% of total food) early in this century (Blegvad 1916, 1930) and of moderate importance from 1950 to 1964 (Bagge 1978). Investigations in Kiel Bay, the North Sea, and the Irish Sea showed that polychaetes were the dominant prey, followed by molluscs. However, in these areas, *P. crassa* was not dominant among polychaetes and brittle stars such as *Amphiura filiformis* were of little importance in the plaice diet (Arntz 1980; Arntz and Finger 1981; Braber and de Groot 1973; Carter et al. 1991). In Kiel Bay, ophiuroids are temporarily rare in the benthos because they cannot propagate there.

Investigations by Blegvad (1916) in the Kattegat showed that echinoderms and crustaceans were selected equally (35% of biomass consumed) by dab and that polychaetes contributed to 20% of the food taken. *Amphiura filiformis* was the dominant species in both Blegvad's and the present study, but the relative importance of this species has increased during the 1980's compared with the beginning of the century. Similarly, high (60%) consumption of *Amphiura filiformis* by dab was found in muddy areas in the southern North Sea (Creutzberg and Duineveld 1986; Duineveld and van Noort 1986). In sandy areas, however, polychaetes, molluscs, and fish dominated in the diet and echinoderms contributed up to 3% of all prey (Creutzberg Duineveld 1986). In the Öresund, *Amphiura filiformis* contributed to 15 and 35% of the biomass consumed by large (24–29 cm) and small (16–20 cm) dab, respectively (Degel and Gislason 1988). A low (4%) level of echinoderms in the diet of dab was also found in Kiel Bay, where low salinity restricts the distribution of ophiuroids (Arntz 1971). Knust (1986) showed seasonal variation in the diet of dab in the German Bight: *Ophiura albida* made up over 60% of the food consumed in winter (October–March) whereas during summer (July–August), up to 80% of the diet was crustaceans. In the present study (September–May), echinoderms were the dominant prey and crustaceans were of little importance in the diet.

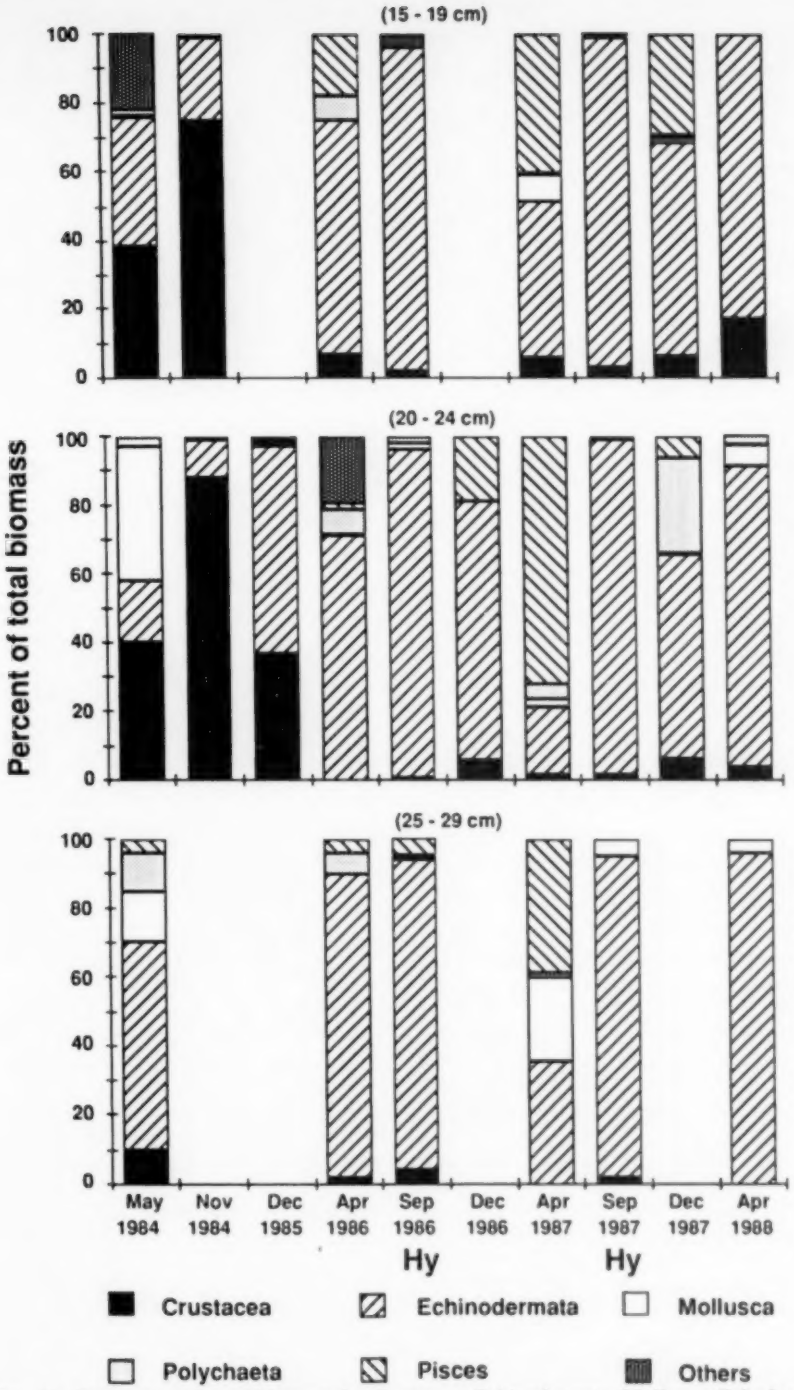

FIG. 11. Percentage of total biomass for Crustacea, Echinodermata, Mollusca, Polychaeta, Pisces, and other prey items in the diet of three length-classes (15–19, 20–24, and 25–29 cm) of American plaice (*Hippoglossoides* platessoides) in the southeast Kattegat during 10 sampling occasions from 1984 to 1988. Hy indicates a period with hypoxia.

American plaice are considered to be crustacean and fish predators (Krüger 1942; Pitt 1973; Arntz 1980; Arntz and Finger 1981), but in some areas, echinoderms (mainly brittle stars) contributed to up to 40% of the gut contents (Scott 1973; Lande 1976). In the present study, brittle stars made up to 66% of the diet (a level not reported in previous

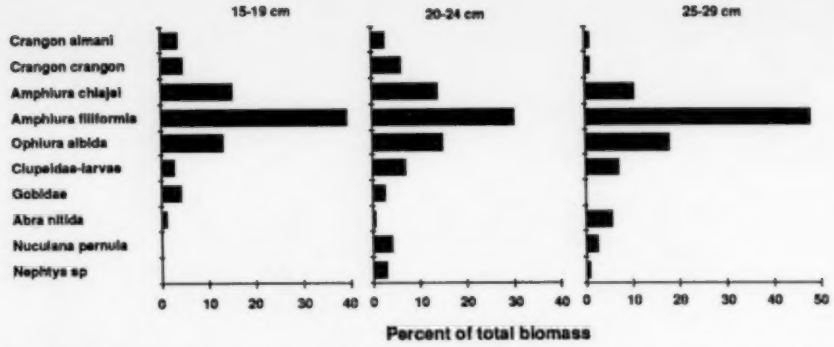

FIG. 12. Percentage of total biomass for the 10 most important prey items in the diet of three length-classes (15–19, 20–24, 25–29 cm) of all American plaice sampled in the southeast Kattegat from 1984 to 1988.

studies) and crustaceans and fish were of minor importance relative to other areas.

Among selective fish predators, dietary composition is determined by prey community structure and availability. The morphology of the mouth and alimentary tract is adjusted for special types of food organisms living in a certain habitat (de Groot 1971), and if the structure of the food resources changes, the diet can only vary within certain limits. Thus, structural changes in benthic communities will be reflected as a shift in the diet of benthic feeding fish, limited by the availability of food items and the morphology of the fish. Recent investigations of the benthos in the Kattegat demonstrated considerable structural changes during this century. In general, large, long-living species have been replaced by smaller species with a shorter life span (Pearson et al. 1985). The most conspicuous change of the benthic community is the drastic increase in the abundance and biomass of *Amphiura filiformis*, presently dominant in benthic communities below the halocline in the Kattegat (Pearson et al. 1985; Josefsson 1990). During the 1970's and 1980's the biomass of the benthic infauna increased by a factor 1.8, mainly as a consequence of higher *Amphiura filiformis* biomass (Josefsson 1990). Furthermore, *Polyphysia crassa* is now the dominant polychaete (in terms of biomass) and overall among the four most dominant macrobenthic species in the study area in the southeast Kattegat (L-O. Loo, University of Göteborg, personal communication), but was not a dominant species in the beginning of this century (Petersen 1918). Observed structural changes in benthic infauna implicate organic enrichment due to increasing eutrophication as a potential cause in the Kattegat (Pearson et al. 1985; Josefsson 1990). Enhanced organic input would favor species like *Amphiura filiformis* and *Polyphysia crassa* (Pearson and Rosenberg 1978), which are the dominant food items for the three flatfish species in this study.

Food Selection in Relation to Oxygen Concentration

Seasonal dietary change is generally attributed to variation in the availability of prey. The distribution of many prey (and thus vulnerability to predation) varies due to migration, dispersion, concentration, or changes in the vertical distribution of different reproductive stages. For example, some infaunal polychaetes become pelagic during spawning, thereby increasing their vulnerability to predation. Seasonal variation in prey distribution may also result from changes in temperature, salinity, light, oxygen, and currents. In the present investigation, oxygen mean concentration in the bottom water showed considerable seasonal variation (0.9–10.4 $mg \cdot L^{-1}$) whereas only smaller changes in temperture (4–9°C) and salinity (32–34‰) have been recorded over the period of investigation (Baden et al. 1990a; Ryberg et al. 1990). Therefore, seasonal variation in fish diet is discussed mainly in relation to oxygen concentration.

Crustaceans were the main food for young Atlantic cod and whiting and were also consumed by older individuals and by American plaice. During hypoxia in September 1986 and 1987, stomachs of small and midsized cod contained lower biomass of crustaceans ($p = 0.01$, Mann–Whitney U-test) than on other sampling occasions. Some crustaceans also showed a decreasing importance for cod, whiting, and American plaice during the investigation (1984–88). For example, the shrimp *Pandalus borealis* was eaten by cod and whiting in 1984 and 1985 but not later during the investigation period. Crustaceans are sensitive to low oxygen and may avoid hypoxic areas (Pihl et al. 1992). During hypoxia in the same area, Norway lobster consumed far fewer crustaceans than in areas without oxygen deficiency (Baden et al. 1990b). In York River, Chesapeake Bay, crustaceans were significantly reduced in the diet of two dominant demersal fish species after hypoxic periods, and polychaetes became dominant (Pihl et al. 1992). In contrast the hermit crab (*Pagurus bernhardus*) was taken in this study only during periods of hypoxia by plaice and American plaice. Crustaceans were normally not eaten by plaice, but during the hypoxic event in September 1987, soft parts of hermit crab constituted 25% of the diet (Table 2). Hermit crab also increased in biomass in small and midsized cod during hypoxia, a pattern which was also seen for Norway lobster caught in the same area (Baden et al. 1990b). Only soft parts were found in the stomachs, suggesting predation on individuals that had emerged from the shell due to low oxygen levels.

Brittle stars, dominated by *Amphiura filiformis*, were the main food for all size-classes of dab and American plaice. The contribution by *Amphiura filiformis* to the total food intake was higher during hypoxia in September 1986 and 1987 than during other sampling occasions for both dab and American plaice ($p = 0.010$ and 0.015, Mann–Whitney U-test). The three brittle stars *Amphiura filiformis, A. chiajei,*

and *Ophiura albida* made up over 95% of all food taken by American plaice in September 1986 and 1987. During the hypoxic events, increased dominance of these brittle stars was also found in the diets of plaice and cod. Cod did not consume echinoderms during normoxic conditions. Another echinoderm, *Echinus esculentus*, was only consumed during hypoxia in September 1986 and 1987 and was found in stomachs of cod, dab, and American plaice.

Polychaetes were the main food for plaice during most sampling occasions. However, during hypoxia in September 1986 and 1987 the importance of polychaetes decreased and instead, *Amphiura filiformis, Pagurus bernhardus, Abra nitida,* and *A. alba* were eaten (Table 2). In contrast, the polychaete *Aphrodite aculeata* was eaten by midsized cod during hypoxia and in September 1987 constituted 43% of their diet.

During normoxic conditions, infaunal species are to some extent protected from fish predation by the sediment. The probable explanation for the high consumption by demersal fish of deep-burrowing species during hypoxia is that these animals emerge from the sediment when oxygen is depleted (Jørgensen 1980). In field video-camera investigations in the Kattegat and in an experimental study, some dominant infaunal species typically found in the southeast Kattegat moved to the sediment surface at oxygen concentrations of about 2 $mg \cdot L^{-1}$ (Rosenberg et al. 1991). Bivalves extended their siphons 20–30 cm into the water column, and brittle stars could be seen actively moving the arms (*Amphiura filiformis*) or standing on arm-tips with their body-disk elevated (*Ophiura albida*) to reach higher oxygen concentrations. Both bivalves and brittle stars survived for several days in oxygen concentrations of 1 $mg \cdot L^{-1}$. This behavior exposes individuals to greater predation risk. Oxygen consumption in the study area mainly (to 90%) takes place in bottom water (Rydberg et al. 1990), and a sharp oxygen gradient normally develops over the sediment surface (Jørgensen 1980; Rosenberg and Loo 1988). In 1986 and 1987 the hypoxia extended about 1 m above the sediment, and even though the demersal fish biomass was reduced to 25% of that found during normoxia, the remaining fish could exploit the vulnerable emerging benthic infauna. Some fish could also remain above hypoxic bottom water and periodically swim to the bottom for foraging. A similar pattern of benthic infaunal exploitation during hypoxia was seen in York River, Chesapeake Bay, where large, deep-burrowing polychaetes were consumed by demersal fish during and immediately after hypoxic events, resulting in a significant increase of the prey size (Pihl et al. 1992). During the severe hypoxia in the Kattegat in September 1988, very few fish were caught in the trawl; instead, large quantities of benthic infaunal organisms were caught. Most of these were moribund with a biomass estimated between 200 and 1600 kg (wet weight)·1-h $trawl^{-1}$, which biomass exceeded normal values for infauna by one to two orders of magnitude (Baden et al. 1990a).

The results indicated that a general long-term change in the diet of dominant bottom-feeding fish in the Kattegat has taken place since the beginning of the century, and for some of the species the feeding pattern is different from that in adjacent areas. The observed dietary shift is suggested to be due mainly to structural changes in benthic macrofauna. Generally, prey species favored by increased organic enrichment are now dominant in fish diets. Further, this study demonstrates reduced consumption of epibenthic crustaceans, which are sensitive to low oxygen, and increased consumption of more hypoxia-tolerant infaunal species during periods of low oxygen concentration. During periods of moderate hypoxia the biomass of the demersal fish was reduced, but part of the populations could take advantage of the increasing food resources when infauna emerged from the sediment. However, during severe hypoxia, fish were almost absent and the benthic infauna were found dead or dying on the sediment surface (Pihl 1989; Baden et al. 1990a; Rosenberg et al. 1992).

The observed shift in the diet from crustaceans and molluscs to polychaetes and echinoderms may imply a general decrease in the nutritional value of the food taken. The energetic value of the brittle star *Amphiura filiformis*, which was dominant during hypoxia for most of the study species, is about 50% of the value of crustaceans and molluscs per unit biomass (Brey et al. 1988). If the fish cannot compensate for the reduction in energy intake with an increased consumption, their growth will be negatively affected. Under laboratory conditions, growth of plaice and dab was significantly reduced when exposed to levels of 30–50% oxygen saturation (personal observation). From field investigations in the Kattegat, a continuous general decrease in the average size of plaice and dab has been observed during the 1980's (Bagge et al. 1990; Ulmestrand 1992), which could be a consequence of both a direct stress from hypoxia and a decrease in the energy content of the food resources.

This study emphasizes the potential effects of organic enrichment and hypoxia, as a consequence of eutrophication, on trophic interactions in marine benthic communities. Both long- and short-term dietary changes were observed among dominant demersal fish species mainly as a result of structural changes in the benthos. Seasonal hypoxia may partly reduce recruitment, and in severely affected areas in the Kattegat, dominant benthic species have been extinct for some years (Rosenberg et al. 1992). Repeated stress from hypoxia might favor small-sized benthic species with a short life cycle which would in turn favor small-sized fish. Thus, altered food resources and the direct effects of hypoxia might result in a shift in dominance among demersal fish species.

Acknowledgments

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Seasonal Effects of Bleached Kraft Mill Effluent on Reproductive Parameters of White Sucker (*Catostomus commersoni*) Populations of the St. Maurice River, Quebec, Canada[1]

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Gagnon, M.M., J.J. Dodson, P.V. Hodson, G. Van Der Kraak, and J.H. Carey. 1994. Seasonal effects of bleached kraft mill effluent on reproductive parameters of white sucker (*Catostomus commersoni*) populations of the St. Maurice River, Quebec, Canada. Can. J. Fish. Aquat. Sci. 51: 337–347.

Reproductive parameters and accumulation of chlorophenolic compounds by white sucker (*Catostomus commersoni*) populations exposed to bleached kraft mill effluent (BKME) were studied in the St. Maurice River, Quebec. Compared with unexposed populations from the same waterway, exposed white sucker showed obvious effects of chemical exposure as far as 100 km downstream of the mill, as revealed by a strong induction of ethoxyresorufin *O*-deethylase (EROD) activity. In males, circulating plasma testosterone levels were the same at all sites, while 11-ketotestosterone levels were significantly lower at exposed stations. In females, testosterone and 17β-estradiol levels were significantly reduced at the two exposed stations relative to the reference station. Despite different hormone levels during sexual maturation, gonad weight as a proportion of carcass weight was similar at all sites for both sexes during early gonadal development. The relationship between carcass weight and fecundity was more variable at the station immediately downstream of the mill. Biochemical and physiological parameters measured in this study do not allow us to clearly relate perturbations in plasma steroid levels to impaired reproduction as measured by gonad weight and fecundity.

Des populations de meunier noirs (*Catostomus commersoni*) de la rivière St-Maurice, Québec, exposées aux effluents d'une usine de pâte kraft blanchie ont été étudiées. Divers aspects de la reproduction ainsi que l'accumulation de composés chlorophénolés par ces poissons furent évalués. Comparés à une population non-exposée provenant de la même rivière, les meuniers noirs capturés aussi loin qu'à 100 km en aval de l'usine démontrent des effets évidents d'une exposition chimique tel que révélé par une activité très élevée de l'ethoxyresorufine *O*-dééthylase (EROD). Chez les poissons mâles, les niveaux de testostérone plasmatique ne variaient pas entre les sites, alors que les niveaux de 11-kétotestostérone étaient significativement plus bas chez les meuniers exposés. Chez les femelles, la testosterone et le 17β-estradiol étaient significativement plus bas chez les populations exposées. Malgré des niveaux hormonaux réduits, le développement des gonades en proportion du poids de la carcasse était similaire durant l'automne. La relation entre le poids de la carcasse et la fécondité était beaucoup plus variable à la station située immédiatement en aval de l'usine. Les paramètres biochimiques et physiologiques mesurés lors de cette étude ne permettent pas de relier les perturbations des hormones stériodes plasmatiques à des troubles de reproduction tel que mesuré par le poids des gonades et la fécondité.

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Recent research in Scandinavia and Canada has demonstrated various impacts of bleached kraft mill effluent (BKME) on fish. Among others, induction of mixed function oxygenase enzymes (MFO) was observed (Andersson et al. 1988; Lindström-Seppä and Oikari 1988). In fish, the oxidative transformation of xenobiotics can be carried out by the cytochrome P-450 family of enzymes or MFOs (Stegeman and Kloepper-Sams 1987). The cytochrome P-450 system

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TABLE 1. Various responses observed in investigations of impacts of BKME on fish. + = increase, 0 = no change, and − = negative effect of BKME exposure on fish relative to reference populations.

	Fourhorn sculpin (*Myoxocephaelus quadricornis*) Andersson et al. 1987	European pearch (*Perca fluviatilis*) Sandström et al. 1988	European pearch (*Perca fluviatilis*) Karas et al. 1991	White sucker Munkittrick et al. 1991	Redbreast sunfish (*Lepomis auritus*) Adams et al. 1992
Length at age	−	+	−[a]	−	+
Weight at age	−			−	
Growth rate		+		−	0
Lipid accumulation		+[b]		+	+
Gonadosomatic index		−		−	
Gonad weight versus carcass weight		−		−	
Age at maturity			+	+	
Fecundity			+[c]	−[d]	0
Steroid hormones[e]				− (T), 0 (E)	− (E)
MFO induction	+			+	+

[a]Length of embryos.
[b]Reported by condition factor.
[c]Measured by the total number of roe strings on artificial substrate.
[d]Young females only.
[e]T, testosterone in males; E, 17β-estradiol in females.

plays a central role in detoxification of xenobiotics as well as in the transformation of endogenous substances such as steroid hormones (Lehtinen 1990). Although no direct links have been demonstrated between MFOs and steroid hormones, it had been suggested that induction of MFOs by environmental contaminants could have a severe impact on the biotransformation of steroid hormones in fish and on the reproductive success of exposed populations (Lee 1988). Other effects, such as altered immune responses (Andersson et al. 1988; Larsson et al. 1988), differential growth, and impaired reproduction (Larsson et al. 1988), have also been observed. Because similar chlorine bleaching processes are used in North American pulp mills, various studies have been undertaken in the United States and Canada to establish and describe the impacts of BKME on indigenous fish populations.

Various North American studies describe effects similar to those observed in Scandinavia. Although MFO induction seems consistent in BKME-exposed fish, many responses at the physiological and population level are not consistent and seem to be site specific (Table 1). Investigations of white sucker (*Catostomus commersoni*) conducted by McMaster et al. (1991) and Munkittrick et al. (1991) in Jackfish Bay, Lake Superior, indicated reduced levels of virtually all plasma sex steroids for both sexes, along with reduced gonad size and high MFO activity. A preliminary study of the same species conducted by Hodson et al. (1992) on the St. Maurice River, Quebec, also showed decreased serum testosterone in males, but an increase of this hormone in females, although variations were not statistically significant. Hodson et al. (1992) showed a reduced gonad size for both sexes and an MFO activity related to distance from the mill. Our present extensive survey on the St. Maurice River was undertaken to investigate in detail the effects of BKME exposure on fish populations.

A major bleached kraft pulp and paper mill located at La Tuque, Quebec, on the St. Maurice River produces about 1400 tonnes per day of air-dried product and discharges about 8–10 kg of chlorinated compounds per tonne of product, equivalent to more than 10 tonnes per day of chlorinated chemicals (Hodson et al. 1992). Before being discharged to the river, the effluent receives a primary treatment through a settling basin. No other industries or towns occur along the river until Grand-Mère, Quebec, more than 100 km downstream of La Tuque (Fig. 1). Hydroelectric dams present at 0 and 100 km downstream from the mill control log floating and limit fish movements upstream and downstream of the mill. Thus, single industry contamination combined with limited fish movements provides a unique opportunity to study the effects of BKME on fish populations.

Our major objective was to establish whether a correlation exists between the induction of the MFO system and reproductive impairments coincident with decreased steroid levels by comparing one unexposed population of white sucker located upstream of the mill with two BKME-exposed populations located downstream of the mill.

Methods and Materials

Methodological Approach

White sucker is one of the most studied species in environmental investigations of pulp mills in North America. It is common throughout rivers and lakes of Canada and the northern United States, but is not heavily exploited by either sport or commercial fisheries and is consequently not affected by overfishing. Because they are bottom feeders, white sucker are directly exposed to contaminated sediments and are thus likely to exhibit responses to environmental toxicants. Their well-documented biology and the number of other studies of their response to BKME make the white sucker an ideal species for this study (Munkittrick and Dixon 1989).

Evidence of exposure to BKME among the three groups of white sucker was assessed by analysing tissue samples for the presence and concentrations of chlorophenols and chloroguaiacols, compounds typical of BKME. To ensure

that environmental contamination was due mainly to BKME, tissue levels of polychlorinated biphenyls (PCBs) and several organochlorine insecticides not usually found in BKME were also determined.

Induction of the liver MFOs as measured by ethoxyresorufin *O*-deethylase (EROD) was evaluated and related to chemical exposure. Circulating plasma levels of testosterone and 11-ketotestosterone in males and 17α,m20β-dihydroxy-4-pregnen-3-one (17α,20β-diOHprog), testosterone, and 17β-estradiol in females were monitored to evaluate the possible interaction of MFOs and steroid hormones at various stages of reproductive development. Sexual maturation was monitored by measuring gonad weight in relation to body weight of males and females and fecundity of females. We expected the downstream groups of white sucker to exhibit elevated levels of chlorophenolic contamination, greater induction of EROD activity, lower levels of steroid hormones, and retarded gonad development relative to white sucker sampled upstream of the mill.

Sampling Sites

White sucker were sampled with gill nets in the St. Maurice River, Quebec (Fig. 1) during May 8–20 (spawning), August 1–10, and November 13–22, 1990, and May 6–17, 1991 (spawning). At each sampling period, fish were collected at three sites. The reference station 1 (5 km upstream of the mill) is isolated from the downstream stations by a hydroelectric dam. In this part of the river, current is slow and the bottom is covered with bark from floating logs. Station 2, located immediately downstream of the mill, had a faster current and a bottom that was a mixture of bark and sand. Station 3, 95 km downstream of the mill, resembled station 1. There was continuous log floating at all three stations. At each sampling period, temperature was recorded daily and did not differ from one site to another.

At all three stations, the nets were set in 2–5 m of water, in areas with a relatively slow current. Gill nets were composed of three 50-m panels of 8.9-, 11.4-, and 12.7-cm stretched mesh. Fish were removed from the nets at least once a day and kept alive in an aerated tank on the boat. Fish were transported to the land-based laboratory within 30 min for sample collection.

Sample Collection

For the first 30 fish sampled alive at each station, a blood sample was taken by caudal puncture, allowed to clot on ice for 15 min, centrifuged, and the serum quick-frozen in liquid nitrogen for subsequent hormone analysis. The liver was removed, rinsed with an ice-cold solution of 0.15 M KCl, cut into small pieces, and frozen in liquid nitrogen. The length of each fish and weights of the whole body, gonads, liver, intestines, and carcass (whole body minus viscera) were recorded. The first pectoral fin rays were kept to age the fish. Each carcass was immediately frozen on dry ice for contaminant analysis. In the spring, ovaries were immersed in Gilson's solution (Bagenal 1978) for evaluation of fecundity. Fish that had ovulated eggs in the body cavity were not used to evaluate fecundity or steroid levels.

Sample Analyses

Fish carcasses were kept frozen at −20°C until homogenization. The carcass was passed through a Hobart meat grinder three times to ensure a homogenous mix and the homogenate was frozen. The grinder was thoroughly cleaned with soap and rinsed with water and acetone after homogenization of each fish.

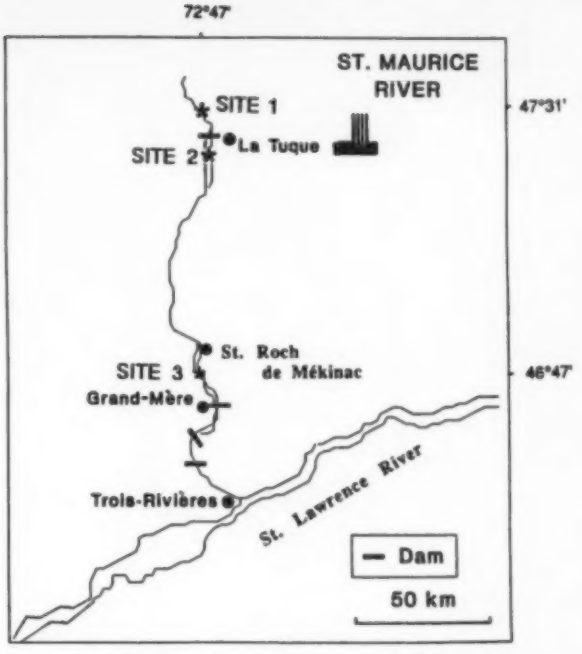

FIG. 1. St. Maurice River and stations sampled in 1990 and 1991. A pulp mill is located at La Tuque and hydroelectric dams at La Tuque and Grand-Mère. Site 1 is 10 km upstream from the pulp mill; sites 2 and 3 are 2 and 95 km downstream of the mill, respectively.

Carcasses of 10 males and 10 females from station 3 were analysed for concentrations of chlorophenolic compounds to test for possible differences of contamination between sexes. No significant differences were found in contamination levels between sexes: for example, 4,5,6-TCG was 1.52 ± 0.51 ng·g wet weight^{-1} for males and 0.99 ± 0.33 ng·g^{-1} for females. Therefore, sexes were pooled for all other analyses. For subsequent analyses, two subsamples of homogenates of 5 g each originating from two individuals of a similar origin, age, and length were pooled together to better estimate the average contamination of the different fish populations. Fish that could not be paired in this fashion were analysed individually. Although pooling samples prevents evaluating individual variability in contamination, our only intent was to discriminate between exposed and unexposed populations according to the concentration of phenolics. Extraction, cleanup, and gas chromatography methods were carried out according to Carey et al. (1988). Analyses of PCB isomers and organochlorine insecticides followed the method of Comba et al. (1989), using a pool of 10 fish for each station. The detection limit of the gas chromatograph was 0.1 ng·g wet weight tissue^{-1}, the percent recoveries of standards varied between 80 and 100%, and the variability of analysis for samples was <12% (six samples compared). Contamination data reported here were not corrected for percent recovery.

TABLE 2. Organochlorine pesticides and ΣPCBs (ng·g wet weight^{-1}) in white sucker from the St. Maurice River. ΣPCBs represents the summation of 137 isomers monitored; n = 10 per station; — = not detected.

	Station 1	Station 2	Station 3
HCB	0.7	1.4	0.7
Heptachlor	—	—	—
Aldrin	—	0.9	0.9
o,p-DDE	—	—	—
p,p-DDE	2.9	2.6	1.9
o,p-DDT	0.3	0.4	—
p,p-DDT	—	—	—
o,p-DDD	3.3	3.0	1.1
p,p-DDD	3.5	5.0	2.0
Mirex	0.4	0.9	0.2
α-BHC	0.8	1.5	0.9
β-BHC	0.1	—	—
δ-BHC	0.6	0.7	0.4
Heptachlor epoxide	1.9	1.8	0.9
δ-Chlordane	0.8	0.8	0.4
α-Endosulfan	1.1	0.9	0.5
α-Chlordane	1.5	4.5	2.5
Dieldrin	3.0	4.0	2.0
Endrin	1.3	2.0	0.6
β-Endosulfan	2.1	2.0	0.8
Methoxychlor	5.5	6.0	5.9
ΣPCBs	4.7	5.3	4.0

Liver samples were kept at −80°C until analysed for enzymatic activities, which was done within 2 wk of sampling. In the laboratory, samples were thawed on ice and analysed for EROD activity in the S-9 fraction of the tissue homogenate, following the spectrofluorometric method described in Hodson et al. (1991).

Blood samples from the caudal artery were collected in May, August, and November 1990 and kept at −80°C until analysed. Following ether extraction of the plasma, 17α,20β-diOHprog, testosterone, 17β-estradiol, and 11-KT were measured by radioimmunoassay (RIA). A description of the antisera used to measure 17α,20β-diOHprog, testosterone, and 17β-estradiol is given in Van Der Kraak et al. (1984), and the 11-KT antisera is described in Wade and Van Der Kraak (1991). All samples were assayed in duplicates and the interassay variability was less than 8%.

Gonads were soaked in Gilson's solution for 2 wk to ensure hardening of eggs and dissolution of ovarian tissue. They were subsequently dried in a low-temperature oven for 24 h at 60°C. Three subsamples were counted and weighed to provide an estimate of fecundity for females of different sites. The dry weights of individual eggs, as calculated by the dry weight of gonad divided by the fecundity counts, were compared using an ANOVA.

Because transformation did not normalize the data set, statistical comparisons of contamination levels, EROD, and steroid levels were done using a Kruskal–Wallis test followed by nonparametric multiple comparisons with unequal sample sizes. Regressions of gonad weight to carcass weight and of fecundity to female carcass weight were compared among sites using a covariance analysis with carcass weight as a covariable and station as a classification factor. When ANCOVA revealed parallel slopes, the analysis was pursued by contrasts on adjusted means to detect any differences between stations. When the analysis revealed nonparallel slopes, a posteriori tests were performed to identify which slopes differed from the others. Normality of data sets was verified by a Sharpio–Wilk test and homogeneity of variances was checked by a Bartlett test before applying the covariance analysis (Zar 1984).

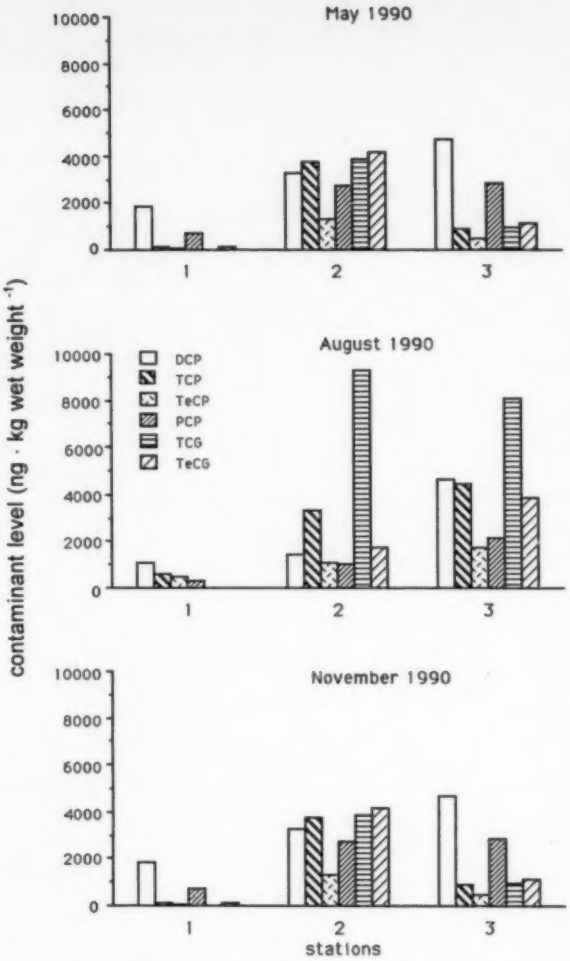

FIG. 2. Chlorophenol and chloroguaiacol levels in white sucker from the St. Maurice River in 1990. For stations 1, 2, and 3, n = 67, 37, and 53, respectively. DCP, dichlorophenols (2,4-DCP, 3,4-DCP); TCP, trichlorophenol (2,4,6-TCP, 2,4,5-TCP, 2,3,4-TCP, 2,3,5-TCP); TeCP, tetrachlorophenol (2,3,4,6-TeCP); PCP, pentachlorophenol (1,2,3,4,5-PCP); TCG, trichloroguaiacol (3,4,5-TCG); TeCG, tetrachloroguaiacol (2,3,4,6-TeCG).

Results

White sucker from the three stations exhibited minor contamination by organochlorine insecticides (Table 2). Contamination in the carcasses of white sucker from all sites varied from lower than the detectable level (0.1 ng·g wet weight flesh^{-1}) to a maximum of 6.0 ng·g wet weight^{-1} for methoxychlor. Some PCB isomers were also present in the fish carcasses, although the levels were very low. Isomer

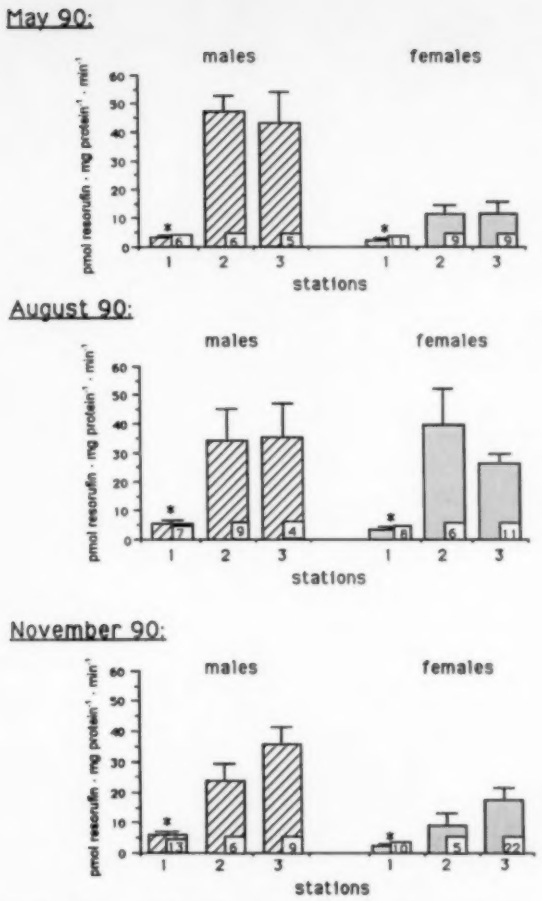

FIG. 3. EROD activity in white sucker sampled in spring, summer, and fall 1990. Error bars represent standard errors. Sample sizes (n) are given in the lower right corner of the histograms; *difference ($p < 0.05$) between reference and exposed stations.

153, which is often the most abundant in biota (Ballschmiter et al. 1981), was the dominant PCB isomer, with a maximum recorded level of 0.05 ng·g wet weight^{-1}.

Fish sampled downstream of the mill had higher ($p < 0.05$) tissue levels of chlorophenols and chloroguaiacols than fish sampled upstream (Fig. 2). The levels of contamination at stations 2 and 3 were generally similar. Comparisons over time revealed that the low level of contamination at station 1 was similar throughout the year ($0.12 < p < 0.86$). Levels of chlorophenolic compounds at stations 2 and 3 were generally similar throughout the year, with the exception of TeCP, TCG, and TeCG at station 3 which were found at higher levels in August relative to May ($0.03 < p < 0.001$). The high levels of TCG and TeCG at stations 2 and 3 allowed us to conclude that fish populations were exposed to BKME and to associate observed effects with BKME exposure. It was not intended in this study to relate a specific level of contamination in a few fish to specific effects on populations.

There were no relationships among the BKME-related contaminant level (taken individually or as a summation) in the carcass and length, whole-body weight, carcass weight, or age (data not shown).

Male and female white sucker sampled downstream of the mill exhibited higher levels of EROD activity ($p < 0.001$) than fish from the reference station (Fig. 3). In August only, enzyme induction in female white sucker followed the same patterns and levels as in males. Among BKME-exposed female white sucker, induction was lowest in May and November, compared with males sampled at all times or with females sampled in August.

Steriod hormone levels of males and females sampled in May 1990 were highly variable, partly due to the spawning activity which causes major and rapid steriod changes during final gonadal maturation (Scott et al. 1984). Gonad development of white sucker sampled on the spawning ground was not perfectly synchronous, which may also contribute to the high variability of sex steroid levels observed in May 1990. In August, both males and females were reproductively inactive (GSI = gonad weight divided by carcass weight <3.0 for both sexes) at all stations resulting in very low levels of hormones. Due to reduced hormone levels in August, no site differences were observed. Because of these confounding factors, data from May and August were unsuitable for comparisons among sites.

In November, white sucker were maturing sexually for spring spawning, and steroid levels were elevated and homogenous in the different populations, permitting valid comparisons among sites. At this time, plasma sex steroid levels were altered in BKME-exposed fish relative to the reference ones. In males, testosterone levels were similar at all sites in November 1990 ($p = 0.150$) (Fig. 4), while 11-ketotestosterone serum levels were reduced at exposed sites ($p = 0.0048$). In females, 17α,20β-diOHprog levels were higher at exposed stations ($p = 0.014$). In contrast with males, female white sucker exposed to BKME exhibited a significant reduction in testosterone ($p = 0.014$) and 17β-estradiol ($p = 0.001$) levels relative to females from the reference site (Fig. 5). For both sexes, no significant correlations between MFO induction and any steroid levels were found ($p < 0.05$).

Male white sucker sampled in November showed no differences among sites in the linear regressions relating gonad and carcass weight ($p = 0.95$, intercept: $p = 0.61$; data not shown). In the following spring, however, male white sucker from different stations had different gonad to carcass weight relationships ($p = 0.0002$) (Fig. 6), with the slope of site 1 being different than the slope of site 2 ($p = 0.013$). The slope at site 3 was not statistically different from that observed at sites 1 and 2. Given the fact that slopes were significantly different and r^2 values were low, statistical comparisons of means were not pursued.

Despite lower levels of hormones at downstream stations in November 1990, all females exhibited a similar gonad weight for a given carcass weight (slopes: $p = 0.93$, intercept: $p = 0.59$; data not shown). In May 1991, females still exhibited comparable gains in gonad weight relative to carcass weight (i.e., parallel curves with $p = 0.15$) (Fig. 7A). However, differences were observed in the elevations of the curves (i.e., the y-intercept was different, $p = 0.002$), which indicates that, on average, some female white sucker had a larger gonad weight for a given carcass weight. A posteriori tests on adjusted means revealed that reference site 1 was different from the exposed site 3 ($p = 0.0005$), with fish from

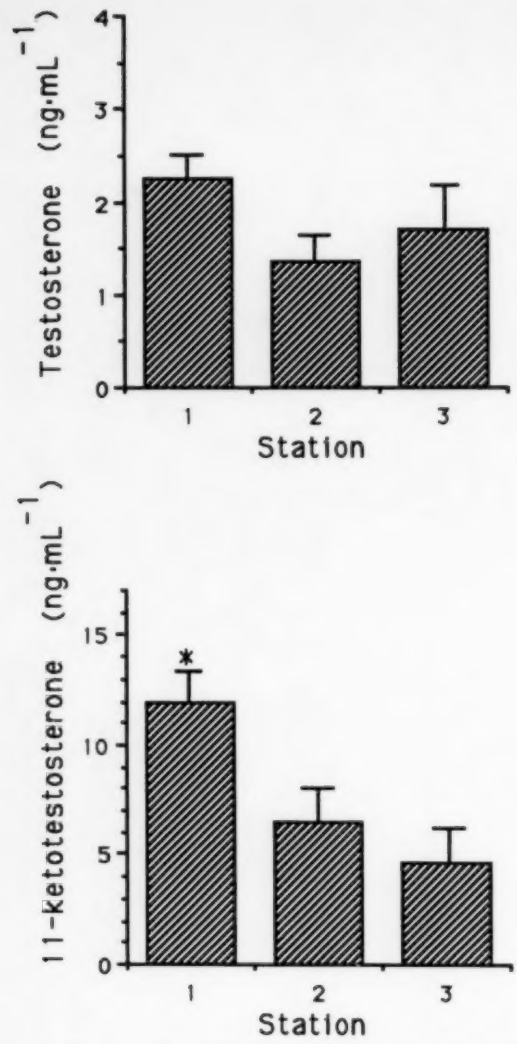

FIG. 4. Testosterone and 11-ketotestosterone blood serum levels in male white sucker from the St. Maurice River in November 1990; $n = 5$, 6, and 8 for stations 1, 2, and 3, respectively. Error bars represent standard errors; *difference ($p < 0.05$) between reference and exposed stations.

site 1 having a higher gonad weight for a given carcass weight. Females from exposed site 2 were intermediate between sites 1 and 3, and therefore did not differ from other sites ($p > 0.14$) in gonadal development (Fig. 7B).

Significant carcass weight related fecundity differences were observed among the three stations the following spring (May 1991) just prior to spawning (Fig. 8). A multiple comparison procedure showed that reference site 1 and contaminated site 3 were different from contaminated site 2 ($p = 0.001$). Females at site 2 showed the greatest variability in the weight–fecundity relationship with carcass weight explaining only 22% of the variation in fecundity. The dry weight of individual eggs, as calculated by the dry weight of gonad divided by the fecundity counts, was not different among stations ($p = 0.17$).

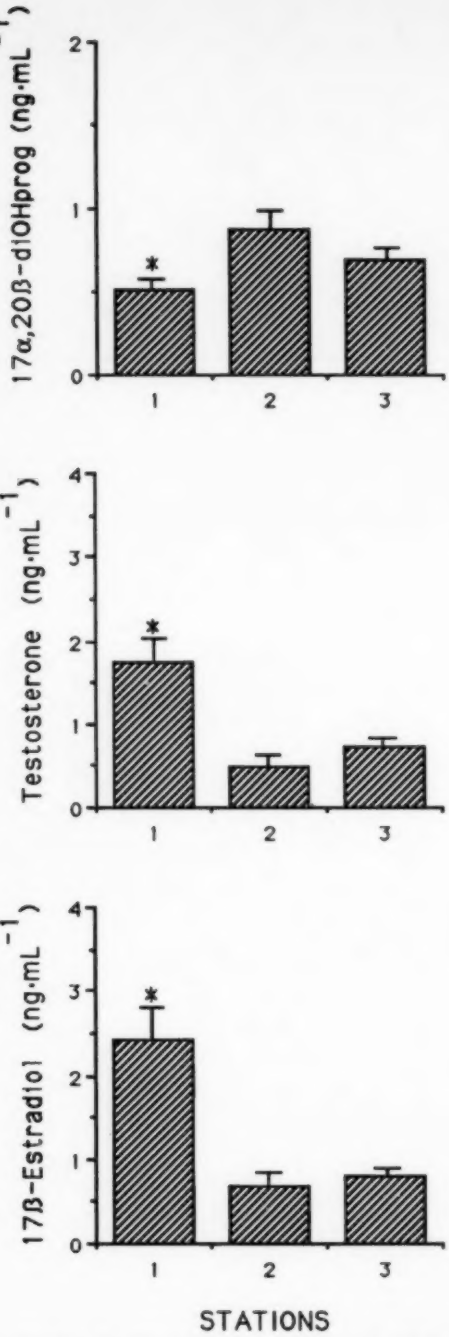

FIG. 5. 17α,20β-diOHprog, testosterone, and 17β-estradiol blood serum levels in female white sucker in November 1990; $n = 16$, 6, and 23 for stations 1, 2, and 3, respectively. *difference ($p < 0.05$) between reference and exposed stations.

Discussion

Contamination of white sucker populations from the St. Maurice River by organochlorine insecticides may be

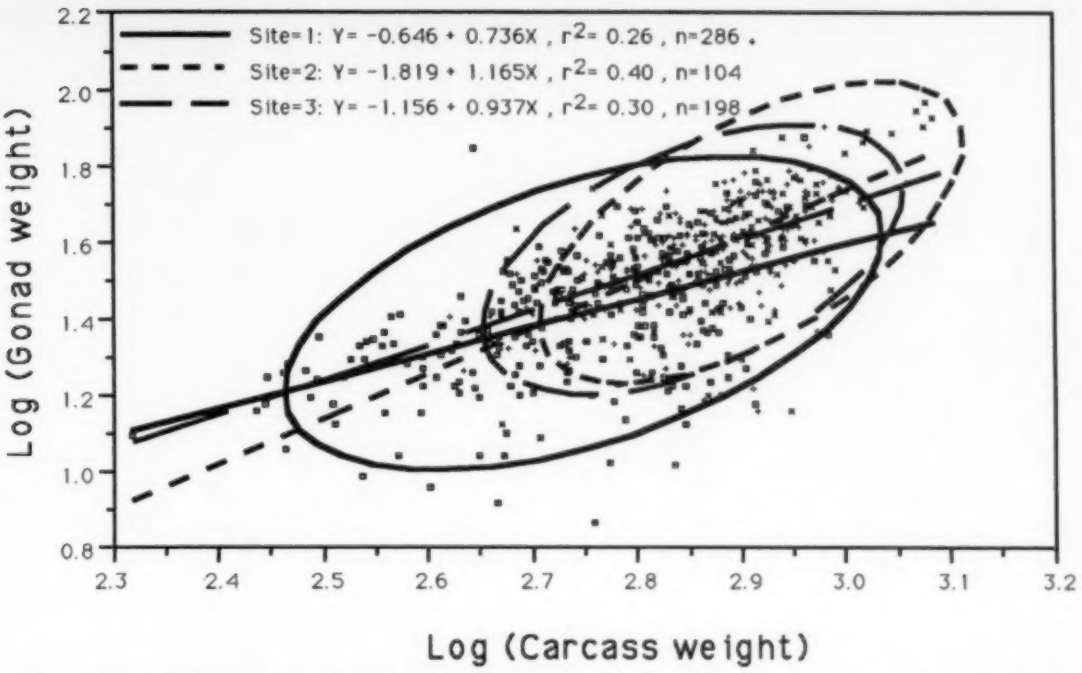

FIG. 6. For male white sucker sampled in May 1991, regressions between the logarithm of gonad weight to the logarithm of carcass weight indicate differences between sites 1 and 2 in the gain of gonad weight relative to a gain in carcass weight ($F = 3.67$, $p = 0.0259$). □, reference site 1; ×, site 2; +, site 3. Ellipses include 95% of data points at each site.

considered negligible when compared with fish from other locations. Trépanier (1984) described PCB concentrations in white sucker originating from other major tributaries of the St. Lawrence River; PCB concentrations were rarely lower than 1000 ng·g wet weight^{-1}. In our study, the low contamination by organochlorine insecticides was similar at all locations, indicating a low level diffuse or point source upstream of the reference station. It is not known if such background contamination would have any biological effects on fish on a long-term basis. Considering that organochlorine compounds such as DDT metabolites and PCB isomers are bioaccumulated rather than metabolized and/or excreted (Paasivirta et al. 1985), low levels of those compounds indicate a chronic, low-level exposure. There is no long-term laboratory study known to us that describe adverse effects related to such a low level of contamination. We therefore considered that biological effects observed in white sucker from the St. Maurice River were mainly due to BKME exposure.

Chlorophenols and chloroguaiacols, indicators of BKME contamination, were found in white sucker from all sites. Levels observed at the reference site were much lower than at downstream sites. Despite their biodegradability, chlorophenolic compounds originating from chlorine bleaching processes have some accumulation potential (Paasivirta et al. 1985). Dichlorophenols were the most abundant phenolic compounds found at the upstream station. Other studies on chlorophenolic compounds in areas free from pulp and paper mills indicate that some chlorophenols may originate from air transport (Paasivirta et al. 1985). This may be the case for dichlorophenols because station 1 is located 10 km upstream of the pulp mill. Fish from stations 2 and 3 are similar in their contamination levels, suggesting that individuals inhabiting those two stations are exposed to similar BKME concentrations with little dilution downstream.

In May 1990, MFO activity among females collected at exposed sites was statistically greater than that of females collected at the reference site. However, in females, reduced MFO levels in May relative to August may be related to the spring spawning activity, when steroid hormones can depress MFO levels. Stegeman et al. (1982) showed that administration of 17β-estradiol in immature brook trout (*Salvelinus fontinalis*) depressed levels of hepatic cytochrome P-450. Pajor et al. (1990) also showed that 17β-estradiol causes a strong suppression of microsomal P-450 content in brook trout. The presence of 17β-estradiol may thus be responsible for MFO inhibition or inhibition of MFO induction in females. 17β-Estradiol could act through a negative feedback mechanism, but other mechanisms contributing to sex differences in MFO activity could also intervene. In August, hormone levels were lowest relative to May and November, and EROD activities in both sexes were similar. In November, white sucker were undergoing gonad maturation for the spring spawning. Steroid levels were already high and there was a corresponding depression of EROD activity in females. At present, however, a direct link between EROD and steroid hormones has not been demonstrated either in vitro or in vivo.

White sucker collected downstream of the mill showed a clear response to chemical exposure as indicated by a strong induction of the liver enzyme EROD. Induction is one of the most consistent responses to BKME exposure and may indicate the presence of polycyclic aromatic compounds such as chlorinated dibenzo-*p*-dioxins (Hodson et al. 1991).

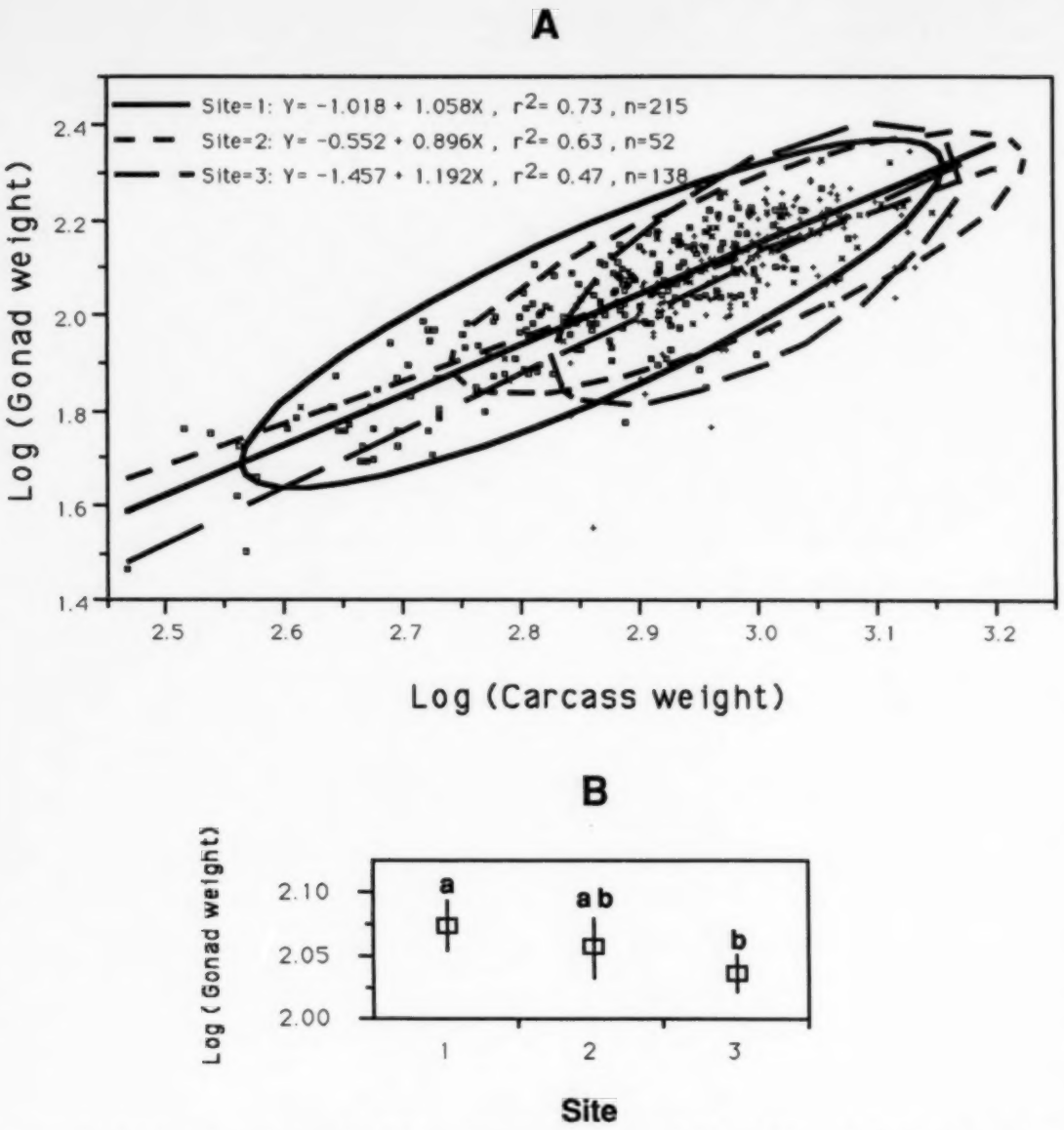

FIG. 7. (A) For female white sucker sampled in May 1991, all slopes are parallel ($F = 1.92$, $p = 0.15$). □, reference site 1; ×, site 2; +, site 3. Ellipses include 95% of data points at each site. (B) Multiple comparisons of adjusted means of the three sites indicate that all stations are not equal. Different letters designate a statistical difference ($p < 0.05$). Error bars represent the confidence interval at 95%.

Dioxins and furans, which have been shown to be strong MFO inducers (Niimi and Oliver 1989), were also detected in carcasses of white sucker from the St. Maurice River in 1989 (arithmetic averages over the three sites of 13.1 pg tetrachlorodioxins·g^{-1} and 107 pg tetrachlorodibenzofurans·g^{-1}, 11 fish pooled, Hodson et al. 1992) and may be partially responsible for MFO induction. Due to the nature of BKME effluent, which contains over 300 components, there may be other products or combinations of products that could act as inducers of cytochrome P-450.

In fish exposed to pulp mill effluents, lower titers of steroid hormones are often observed in conjunction with induced MFO. In this study, similar levels of testosterone were observed in males from all stations in November 1990, suggesting that exposure to BKME did not influence plasma concentrations of this hormone. However, levels of 11-ketotestosterone were lower at BKME-exposed stations. Because 11-ketotestosterone is a metabolite of testosterone in fish (Ozon 1972), lower levels of 11-ketotestosterone suggest a disruption in the male steroid biosynthetic pathway somewhere before 11-ketotestosterone is synthesized (Fig. 4). Impairment could occur at multiple sites, possibly at the

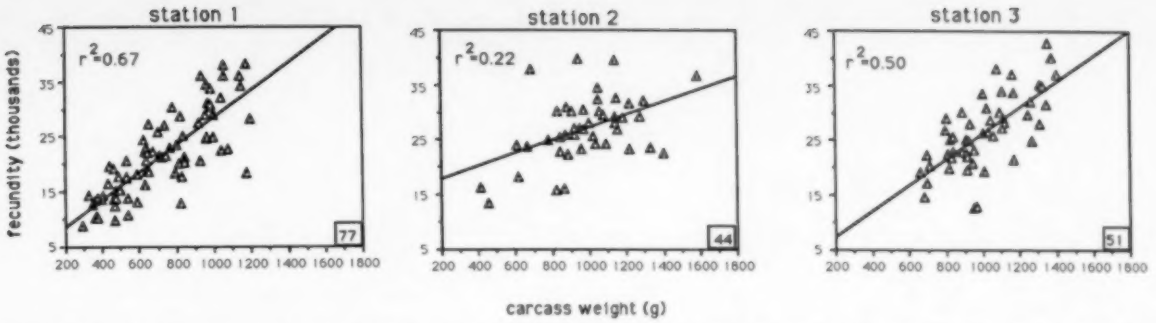

FIG. 8. Fecundity versus carcass weight of females sampled in May 1991. Fecundity varies differently with carcass weight at station 2 relative to stations 1 and 3. Sample sizes are given in the lower right corner of the panels.

level of either of the two enzymes controlling conversion of testosterone to 11-ketotestosterone.

In males, similar testosterone levels at all sites coincided with similar testicular development in November 1990; there was a similar relationship among sites between gonad weight and carcass weight during this sampling period. In the following spawning season of May 1991, however, male white sucker did exhibit differential relationships in the gain of gonad weight relative to the gain in carcass weight, with reference site 1 differing from exposed site 2; exposed site 3 was similar to sites 1 and 2 (Fig. 6). The lack of consistent differences in the observed responses at stations 2 and 3 relative to the reference station contrasts with similar levels of contamination, MFO induction, and 11-ketotestosterone plasma levels observed among male white sucker from the exposed stations. This observation, in combination with the low r^2 values, suggests that variations in gonad weight as a function of carcass weight may be more the result of variations in local environmental conditions than of exposure to effluent.

In females, 17α,20β-diOHprog secretion does not seem to be under a negative influence of xenobiotic effects because circulating levels of this steroid are highest at contaminated sites. However, testosterone, and 17β-estradiol levels were lower in BKME-exposed populations relative to the reference. In females, testosterone and 17β-estradiol are linked, testosterone being the precursor of 17β-estradiol (Scott et al. 1984). Although we know that 17α,20β-diOHprog is involved in the final stage of oocyte maturation immediately prior to spawning (Goetz 1983), we do not know if this hormone plays an active role during early gonadal development. In November, at the reference site, females exhibited a profile of steroid hormones suggesting successful conversion of testosterone to 17β-estradiol.

The limiting factor responsible for a lower level of 17β-estradiol in exposed fish seems to be at least partially due to low testosterone levels, but may also be related to impairment in the biosynthesis of estradiol from testosterone. Low levels of testosterone at exposed sites indicate that a breakdown in the steroid biosynthetic pathway may be occurring somewhere before the synthesis of testosterone in female white sucker. Van Der Kraak et al. (1992) showed that in vitro secretion of testosterone was impaired in eggs originating from BKME-exposed female white sucker. An alternative mechanism explaining differential hormone levels would be increased metabolism of testosterone and 17β-estradiol in exposed females. However, this survey was not designed to differentiate between synthesis and metabolism, but to measure circulating plasma levels. The observed hormonal imbalances may be related to the P-450 system. At present, it is not known if MFO liver enzymes as described by EROD and reproductive steroid hormones are linked in fish. A plausible hypothesis is that both MFO induction and reduced steroid levels are the result of binding by the contaminants to the aryl hydrocarbon (Ah) or to similar receptors, but are not otherwise related (Hodson et al. 1991).

Munkittrick et al. (1991) also observed lowered 17β-estradiol levels in female white sucker from BKME-exposed populations from Lake Superior. However, in contrast with our study, they observed lower levels of testosterone in exposed male white sucker. Hodson et al. (1992) also observed a tendency to decreased testosterone levels in male white sucker from the St. Maurice River in 1989 and slight but nonsignificant increased levels of testosterone and 17β-estradiol levels in BKME-exposed females. However, in that study, fish were sampled in August when steroids were at very low levels. It is unknown whether these different interstudy results reflect seasonal variations in plasma steroid levels or different degrees of impairment.

White sucker undergo gonad maturation in November, but the apparently reduced conversion of steroid precursors to testosterone and 17β-estradiol in females from the St. Maurice River was not reflected in their gonad development during November sampling. Despite lower levels of steroids, females from downstream stations developed gonads to the same extent as reference fish. However, Fig. 6 and 7 indicate that smaller sizes of both males and females are missing from the sample of mature fish, which may reflect an effect of BKME on maturation. Lower levels of testosterone and 17β-estradiol observed in white sucker of the St. Maurice River in November were not necessarily indicative of reproductive impairment during early gonad development of mature fish.

During May 1991, extensive sampling allowed differentiation of gonad development relative to sites. Although all female white sucker exhibited a similar gain in gonad weight relative to a gain in carcass weight in May 1991 (i.e., all curves are parallel), females at reference site 1 had a higher gonad weight than females at exposed site 3 (Fig. 7). We do not know, however, if this small difference in gonad development indicated a biological impact on white sucker populations because fecundity was similar at sites 1 and 3. In addition, fish from site 2 located immediately downstream of the effluent do not appear to be different in their

gonad weight to carcass weight relationships from fish originating from site 1 or 3. Moreover, all fish appeared to have similar mean egg weights. The difference in female gonad development may reflect local environmental conditions prevailing during the reproductive cycle because white sucker are a very phenotypically plastic species (Scott and Crossman 1973).

Although ovarian development was similar at all sites in November of 1990, fecundity differences were observed among the three stations in the following spring. Immediately downstream of the mill, numbers of eggs were poorly correlated with the carcass weight (Fig. 8), while fecundity–weight relationships of fish from reference station 1 and exposed station 3 were statistically similar. The greater variability in the number of developed eggs at site 2 seems to be unrelated to hormone levels, which were altered in a similar fashion at stations 2 and 3. Since only a limited number of reproductive characteristics were measured, it is possible that other mechanisms may be operating.

Despite chemical exposure, enzyme induction, and some reproductive hormonal responses, reproductive impairment was not clearly evident at the level of gonad development, with the exception of increased variability of fecundity–weight relationships immediately downstream of the mill. However, absence of reproductive impairment does not correlate with hormonal variation. In contrast with these results, McMaster et al. (1991) observed that female white sucker exposed to BKME in Jackfish Bay on Lake Superior contained fewer eggs at maturity and exhibited lower gonadosomatic indices, elevated MFO activity, and reductions in plasma steroid levels. In the same area, Munkittrick et al. (1991) also observed delayed sexual maturity and reduced gonad size associated with depressed steroid levels in BKME-exposed white sucker populations. In the same study, however, BKME-exposed female longnose sucker (*Catostomus catostomus*) showed lower steroid levels but similar gonad sizes relative to a reference population. Male longnose sucker from the same contaminated sites did not show any changes in steroid levels or in gonad size compared with a nonexposed population. Munkittrick's and McMaster's study sites were located in a relatively shallow bay of Lake Superior, into which the effluent-carrying creek flows and possibly disperses, creating strong gradients of BKME concentration. The St. Maurice River, however, is a dynamic system in which BKME is rapidly diluted in the river, after which concentrations appear to change little between sites downstream. Therefore, differences in responses of fish between the two study areas may be due to differences in exposure intensity.

The different results from these investigations suggest that elevated MFO activity and altered plasma steroid levels in response to BKME exposure cannot always be associated with obvious reproductive impairment through reduction in gonad size or fecundity. The biochemical and physiological parameters measured in this upstream–downstream study (testosterone, 11-ketotestosterone, 17β-estradiol, 17α,20β-diOHprog, gonad weight, egg weight, and fecundity) do not allow us to clearly relate perturbations in plasma steroid levels to impaired reproduction as measured by gonad weight and fecundity in the St. Maurice BKME-exposed populations of white sucker. However, additional studies are underway to investigate in more detail the effects of pulp and paper mill effluents and natural environmental variations on fish population demographics. Environmental factors may camouflage or modulate fish responses to pulp mill effluents.

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Fourier Analysis of Sea Scallop (*Placopecten magellanicus*) Shells in Determining Population Structure

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Kenchington, E.L., and W.E. Full. 1994. Fourier analysis of sea scallop (*Placopecten magellanicus*) shells in determining population structure. Can. J. Fish. Aquat. Sci. 51: 348–356.

Sea scallops (*Placopecten magellanicus*) were sampled from each of four scallop beds: the northeast peak of Georges Bank, Western Bank, St. Pierre Bank, and the Bay of Fundy near Digby, N.S. The outlines of scallop top shells were captured by video imagery using a semiautomated approach. Fourier shape descriptors were calculated and analyzed. Age and year-class effects were shown to be important factors influencing scallop shell shape. Allometric effects on Fourier harmonics also have a significant influence on shell shape in this species. Comparisons of Fourier harmonics between scallop beds separated by age and sampling year, and corrected for allometry, revealed significant differences in the majority of harmonic numbers. Post hoc analyses identified St. Pierre Bank and the Bay of Fundy as being significantly different from the other beds in a suite of separate analyses.

Des pétoncles géants (*Placopecten magellanicus*) ont été échantillonnés dans chacun des quatre gisements : le pic nord-est du banc Georges, le banc Occidental, le banc St. Pierre et la baie de Fundy près de Digby (N.-É.). On a capté sur film vidéo en mode semi-automatique les contours des coquilles supérieures des pétoncles. On a calculé et analysé des descripteurs de Fourier pour les formes. Il a été montré que les effets de l'âge et de la classe annuelle étaient des facteurs qui influaient grandement sur la forme de la coquille. Des effets allométriques sur les harmoniques de Fourier ont aussi une influence importante sur la forme des coquilles chez cette espèce. Des comparaisons des harmoniques de Fourier des différents gisements de pétoncles séparés par âge et par année d'échantillonnage, et corrigées pour tenir compte de l'allométrie, ont révélé des différences significatives dans la majorité des valeurs harmoniques. Des analyses a posteriori ont montré que le banc St. Pierre et la baie de Fundy différaient énormément des autres gisements dans une suite d'analyses distinctes.

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The sea scallop *Placopecten magellanicus* (Gmelin) occurs only in the northwest Atlantic Ocean from Cape Hatteras north to Labrador (Bourne 1964). Within this area the species is concentrated in persistent, geographically discrete aggregates or "beds", many of which support valuable commercial fisheries. The larger beds are found offshore and in the Bay of Fundy off Digby, N.S. (Fig. 1).

Scallops within different beds are known to differ in somatic and reproductive physiology (e.g., MacDonald et al. 1985a, 1985b; Langton et al. 1987). As a result, there are significant differences in growth rates (Black et al. 1993), and in those shell morphometry characters that are correlated with shell height and weight (e.g., Volckaert et al. 1991), among beds. These pronounced phenotypic differences have been largely ascribed to environmental influences, as such factors as food availability and temperature are strongly linked to growth (cf. Thompson and MacDonald 1991). However, size- and growth-independent shape measures of shell morphology have not previously been described in this species. Shape characteristics are expected to show a different response from size-based characters to environmental stimuli, and thus may reveal different facets of population structure important to the biology of this species.

Size-independent shape characterization can be rapidly and accurately assessed by a method known as Fourier analysis. The Fourier series mathematically compartmentalizes the shape outline into a series of standard sinusoidal components which when summated converge to the empiric shape of the outline. This method has been used to study shape variation in such diverse biological subjects as mussel shells (Ferson et al. 1985; Corkrey 1990), fish otoliths (e.g., Casselman et al. 1981; Bird et al. 1986; Castonguay et al. 1991), fish scales (e.g., Jarvis et al. 1978; Riley and Carline 1982), mice vertebrae (e.g., Johnson et al. 1988), human (Lu 1965) and primate (O'Higgins and Williams 1987) skulls, zooplankton species (Jeffries et al. 1984), and mosquito wings (Rohlf and Archie 1984). The regular outline of the scallop top shell makes it particularly amenable

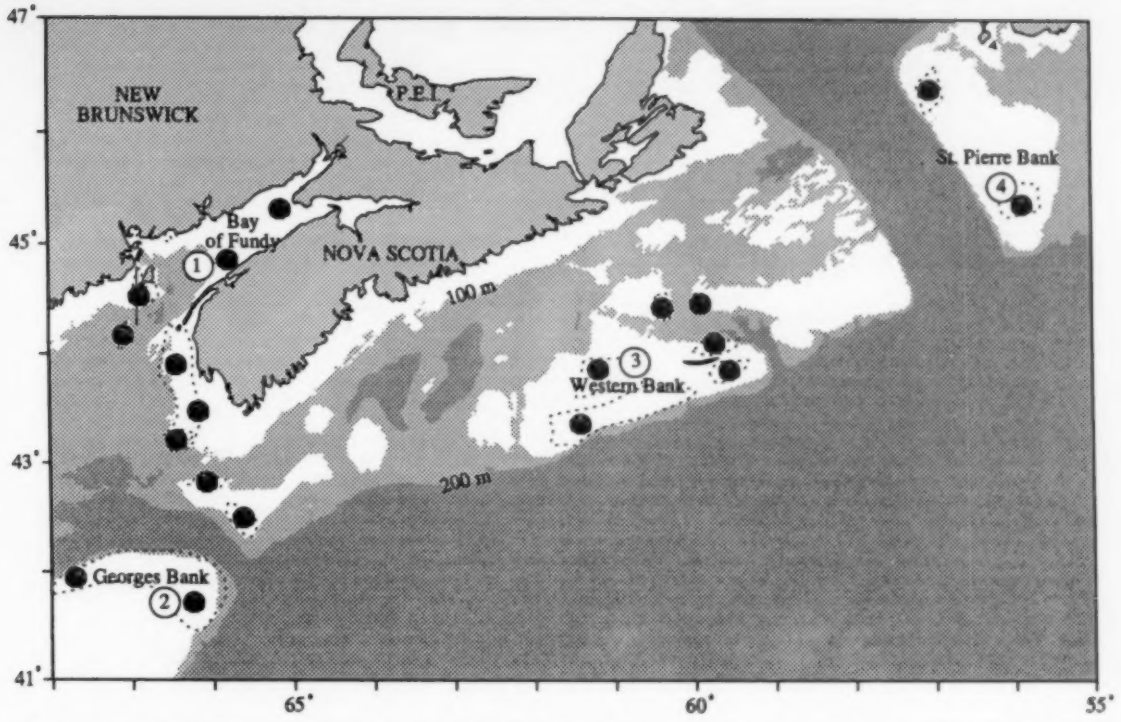

FIG. 1. Distribution of *Placopecten magellanicus* in Atlantic Canada (after Black et al. 1993). Commercial scallop beds are indicated by the scallop shell symbols. Samples were collected from the numbered beds as in Table 1.

to this type of analysis. In this study, aspects of size- and growth-independent shape variation are described for several commercially important scallop beds.

Material and Methods

Sea scallops were randomly sampled from commercial catches from each of four scallop beds (Fig. 1; Table 1): the Bay of Fundy near Digby, N.S., the northeast peak of Georges Bank, Western Bank, and St. Pierre Bank. Commercial catches are made by towing a scallop drag, or in the case of the Bay of Fundy, scallop buckets, for an average period of 30 min over the beds. The distance covered in a single tow can be up to 1 km (G. Robert, Department of Fisheries and Oceans, Halifax, N.S., personal communication). Samples from the Bay of Fundy were all taken from the traditional fishing grounds off Digby. This area has supported a scallop fishery since the 1920s (Sinclair et al. 1985). Twenty tows were made in an area extending from Centreville, south of Digby, to Delap Cove north of Digby, within 20 km from shore. On Georges Bank, there are three main fishing areas. The most productive of these is the northeast peak (Tremblay and Sinclair 1992). In this study, scallops were collected from 33 separate tows made by 17 different vessels that covered the entire area known as the northeast peak. Western Bank scallops were collected from 11 tows made by 11 different vessels. The majority of the tows were made along the 100-m depth contour on the southern edge of the bank over 2° of longitude. In contrast with the others, the St. Pierre Bank sample came from the total catch of a single vessel and thus represents scallops from a number of tows over the southerly edge of the bank.

The ages of the animals were determined by counting annual growth rings (following Bourne 1964) and the top valve (shell) was retained. Only 4-, 5-, and 6-yr-old animals were used in these analyses. The number of animals in each year-class, the mean diameter of the shells in the sample, and the year of capture are indicated in Table 1. Some shells were damaged during collection by the fishing gear or in the sorting procedure. Epoxy was used to smooth minor chips to their original contours. The natural outline of a scallop is regular, permitting such repair to have high precision. Severely damaged shells (shells where the original contours could not be recreated) were discarded.

Fourier Analysis

The images of the scallop top shells were captured using a digitizing video frame-grabber connected to a 512 × 480 pixel CCD video camera. The profiles of the scallops were extracted from the digitized scallop using an edge-detection algorithm first described in Fico (1980). Information on the hardware–software systems used in this study is available from W.E. Full. A minimum of 700–1000 edge-points were defined for each silhouette.

A Fourier series is a mathematical expression consisting of the contributions of sine and cosine forms, the sum of which completely quantifies a single-value curve (cf. Ehrlich et al. 1980; Ferson et al. 1985; Bird et al. 1986). In closed form (i.e., the beginning and end of the curve are the same point), the Fourier transformation partitions the shell image into a

TABLE 1. Sampling scheme and harmonic numbers found to be significantly correlated ($\alpha = 0.05$) with size within location subgroups. Mean shell diameters are calculated from values of the first harmonic (size term).

Location	Age of scallop (yr)	Mean diameter ± SD (cm)	Year of capture	No. of individuals	Harmonic number correlated with size	
					Negative correlation	Positive correlation
Bay of Fundy	4	9.07 ± 0.71	1983	78	2, 3, 4, 17, 18	13
Georges Bank	4	10.02 ± 0.54	1983	308	3, 4	None
Western Bank	4	10.51 ± 0.76	1983	19	2, 3, 4	8
St. Pierre Bank	4	10.77 ± 0.43	1989	42	None	None
Bay of Fundy	5	9.63 ± 0.43	1983	191	2, 3, 11	22
Georges Bank	5	10.94 ± 0.70	1983	270	2, 3, 6, 11, 12, 18, 19	7, 8, 9, 14, 15, 23
Western Bank	5	11.16 ± 1.08	1983	41	2, 3, 5, 6, 11, 12, 18	7, 8, 14, 15
Western Bank	5	9.41 ± 0.44	1984	49	11	23
Western Bank	6	10.51 ± 1.65	1984	28	4	None

series of components (cosine functions) that are each characterized by an amplitude (the contribution of an individual term to the overall shape) and a phase angle (the amount of rotation needed by an individual cosine function to maximize its contribution). The summation of the first six harmonics largely captures the gross shape of the subject. The higher order terms contribute to the finer detail of the outline.

A set of 96 edge-points were interpolated from the original 700+ captured by the edge-detection system. These 96 points were chosen using an algorithm presented in Full and Ehrlich (1982) that preserves homology between shapes and satisfies the various Nyquist criteria. This reduction in the data set negates any effect of video resolution. From these 96 edge-points, 48 harmonic amplitudes and phase angles were calculated. In the discrete Fourier transform used here, the sum of all of the harmonic values at the set of 96 points used to define the shape passes exactly through those points. More edge-points were used than were minimally needed in order to reduce aliasing effects that could bias the subsequent analyses. In this data set, 10–15% of the total variance was tied to harmonics 25–48, indicating that aliasing was a potential bias if fewer edge-points had been used. This high level of energy concentrated in the higher harmonics is unusual and may result from the hinge line of the scallop, with the higher order harmonics allowing for increased definition of this feature of the outline.

Only the first 24 harmonic amplitudes were analyzed. The first harmonic represents the homology error and was driven below 0.0001 by the algorithm described in Full and Ehrlich (1982) and hence is not analyzed further. Harmonics 2–24 were used in the subsequent analyses of shape. Harmonic zero, the diameter of the best-fit circle to the digitized coordinates, was used as a measure of size.

Analyses

In order to be able to relate individual harmonics to their contribution to shell shape, a scallop profile was reconstructed (e.g., Ehrlich and Weinberg 1970) from a randomly chosen individual using the "Graphics" program "PolarPlot" of the Mathematica™ software system (Wolfram 1988). The radius $R(\theta)$ is given by

$$(1) \quad R(\theta) = 1 + \sum_{n=1}^{48} H_n \cos(n\theta - P_n)$$

where n is the harmonic number, H_n is the harmonic amplitude for the nth number, and P_n is the accompanying phase angle for the nth harmonic. The diameter of the average circle is replaced with unity when the profiles are standardized.

Although all of the scallop profiles were standardized to unit size, and within each sample group were selected for constant age and year-class, allometric growth could still result in within-sample group growth-related shape differences that would be reflected in the harmonic amplitudes. In order to eliminate these effects, the correlations between individual harmonic amplitudes and the unstandardized first harmonic amplitude within age and bed subgroups were tested. Where significant correlations ($\alpha < 0.05$) within subgroups were found, linear regression equations were used to standardize these harmonic amplitudes to equivalent values for a 10-cm-diameter shell.

Individual harmonic amplitudes within test subgroups were examined for normality using the Kolmogorov–Smirnov test and for homogeneity of variances using Bartlett's test. Harmonic amplitude means of the bed subgroups were calculated with size-corrected values inserted where appropriate. Differences between these means were tested by univariate Model I ANOVA in a one-factor design. Ten designs were analyzed to test for age, year-class, and location (bed) effects (Table 2). Tests between age-classes within beds used amplitudes not standardized for size, as the effects of allometric growth on shape are a part of the ageing process that should be included in a comparison. All tests were interpreted at a significance level of $\alpha = 0.05$. In the 230 ANOVAs used here, about 12 false significances would be expected. Post hoc comparisons of the group means were performed for each significant ANOVA where more than two sample means were included in the analysis. Contrasts were selected across the sample means in order to determine which subgroups differed (Rodger 1974, 1975).

Results

Interpretation of Harmonics

Reconstruction of the shell outline, based on the summation of consecutive harmonic terms, shows that the essence of the scallop shape is captured in the first 24 harmonics and is reproduced in 48 (Fig. 2). While more than one harmonic number contributes to the delineation of any specific shell feature, some generalizations can be drawn. The lower order harmonic numbers define the gross shape of the shell.

TABLE 2. Designs and results of univariate ANOVA of scallop shell harmonic amplitudes.

Data selection criterion	Factors and factor levels	Harmonic number significantly different at $\alpha = 0.05$
	Location/bed	
4-yr-old scallops[a]	Bay of Fundy Georges Bank Western Bank St. Pierre Bank	3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 17, 18, 23, 24
4-yr-old scallops captured in 1983[a]	Bay of Fundy Georges Bank Western Bank	3, 4, 7, 8, 9, 10, 21, 24
5-yr-old scallops captured in 1983[a]	Bay of Fundy Georges Bank Western Bank	2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24
	Age	
Bay of Fundy	Age 4: 1983 Age 5: 1983	7, 8, 9, 11, 12, 18, 21, 24
Georges Bank	Age 4: 1983 Age 5: 1983	2, 3, 4, 6, 7, 8, 9, 11, 12, 14, 15, 18, 19
Western Bank	Age 4: 1983 Age 5: 1983	3, 7, 14, 22
Western Bank	Age 5: 1984 Age 6: 1984	4, 20
Western Bank	Age 4: 1983 Age 5: 1984	2, 5, 6, 7, 8, 9, 11, 12, 14, 15, 18, 19
Western Bank	Age 5: 1983 Age 6: 1984	3, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 17, 18, 23
	Year-class	
Western Bank	Age 5: 1983 Age 5: 1984	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18

[a]Data size-corrected prior to analysis.

Deviations from circularity such as tendencies toward elongation (harmonic 2), triangularity (harmonic 3), and quadrature (harmonic 4) are defined. Harmonics 5, 6, and 7 position the line of the hinge with respect to the shell value; however, the hinge as a feature is not identifiable until harmonics 8 and 9 are added to the equation. The angles where the auricles meet the valve begin to be shaped as the hinge line is formed but are not fully developed until harmonic 13 is added to the equation. After harmonic 15, all of the shell features are in place, and higher order harmonics refine the profile toward the empiric shape of the shell (Fig. 2).

Mean harmonic amplitude spectra from each of the four scallop beds are illustrated in Fig. 3 for 4-yr-old animals. There are three prominent areas of information displayed in these harmonic spectra. The first peak is created by high amplitudes for harmonics 2, 3, and 4. The shells show prominent shape deviations from circularity, in particular, toward elongated (harmonic 2), triangular (harmonic 3), and quadratal or square (harmonic 4) shape. The second peak of the spectrum describes the hinge area that first appears as an identifiable region in harmonic 8 and is developed through the addition of harmonics 10 and 11. The small third peak occurring in harmonics 13–16 further develops the angle between the auricles and the valve. Harmonics 17–24 generally refine the shape and smooth its periphery. The gross pattern illustrated in Fig. 3 is characteristic of the scallop shell, and the same general pattern is seen in the equivalent mean harmonic amplitude spectra of 5-yr-old scallops (Fig. 4).

Allometric Growth

In some age and bed subgroups, a number of the harmonic amplitudes were correlated with size, indicating within-age allometric development (Table 1). Only the St. Pierre Bank sample showed no significant correlations. Amongst 4-yr-olds, Bay of Fundy scallops showed the greatest number of correlations. In that area the faster growing animals within the year-class tended to be more circular in profile, as the majority of the correlations had negative slopes. Western and Georges banks showed a similar pattern in some of the lower order harmonics.

Five-year-old scallops showed a very different pattern of correlations from the 4-yr-olds (Table 1). Approximately half of the harmonics were correlated with size in scallops caught on Georges and Western banks in 1983. Harmonics 2, 3, 5, and 6, which describe departures of the overall shape and the hinge position from circularity, all had negative slopes. Thus, the faster growing animals within this year-class tended to be more circular in gross shape, much as with the 4-yr-olds. Harmonics 7, 8 and 9, which clearly define the hinge line, have positive slopes, indicating that faster growing scallops within the year-class also have more

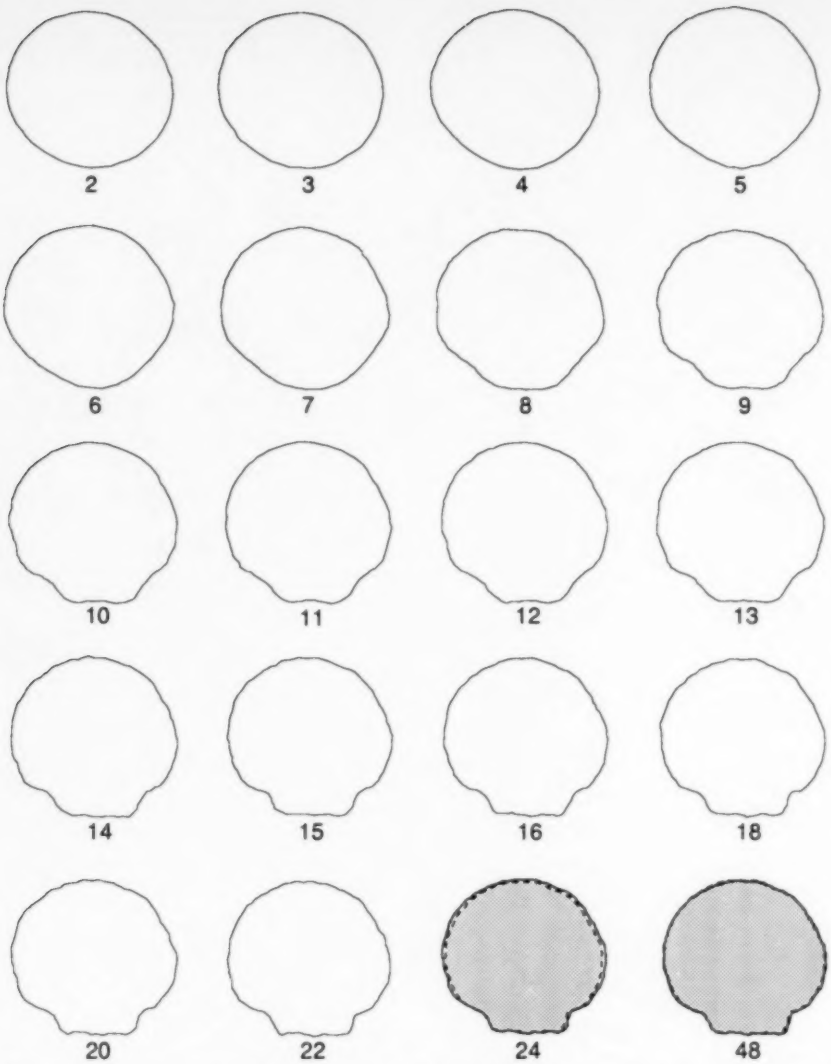

FIG. 2. Shape regeneration of a scallop top shell by addition of successive harmonics computed by the Fourier series (see equation 1). Fourier harmonics were computed for the shaded scallop shape indicated by the broken line. Numbers indicate the number of harmonics added to produce the resultant shape.

prominent hinge lines. Harmonics 11 and 12, which develop and shape the auricles, show negative slopes whereas harmonics 14 and 15, which further refine the auricles, have positive slopes. The harmonic amplitudes that show either positive or negative slopes formed a consistent pattern throughout ages and years of capture (Table 1).

In contrast, both the 5-yr-old scallops from the Bay of Fundy and those collected in 1984 on Western Bank showed relatively few significant correlations between harmonic amplitudes and size (Table 1). The two 5-yr-old year-classes captured on Western Bank differ markedly from each other in this respect. The greatest numbers of significant correlations between the amplitudes and size were seen in the two samples with mean scallop sizes above 10.8 cm in diameter, but there was no consistent trend between mean size and numbers of correlations among the other samples. Thus, the data suggest that there is a change in the shape of scallops as they grow through some critical size between about 10.5 and 11.0 cm in diameter. This critical size cannot be more precisely estimated with the present data set and appears to differ between beds.

Among-Bed and Among-Age Comparisons

All of the harmonic amplitudes were normally distributed and their within-bed variances were homogeneous without transformation. Univariate ANOVA of the size-standardized harmonic amplitudes indicated significant differences in shell shape among 4-yr-old scallops on the various beds (Table 2). When St. Pierre Bank was included in the analy-

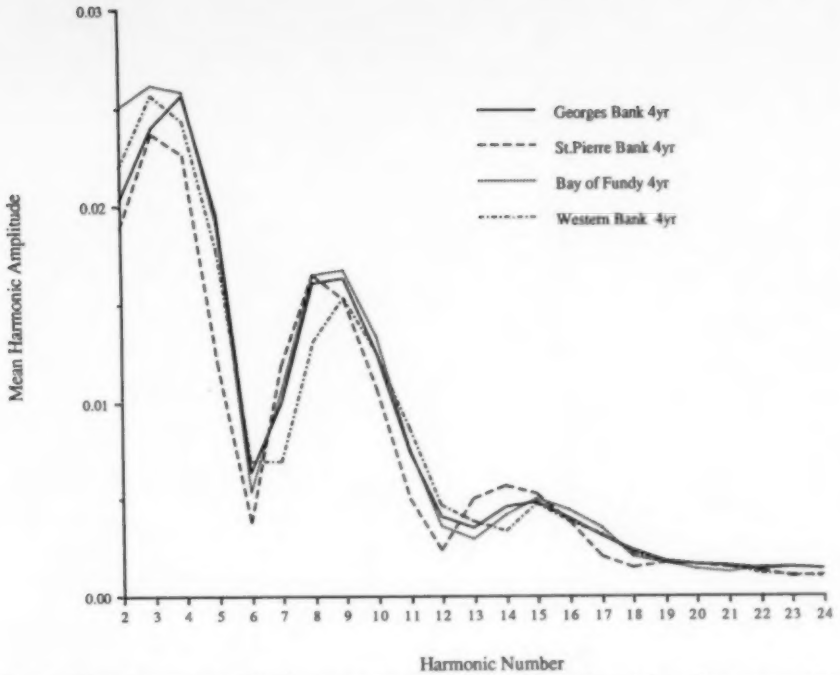
0.03
0.02
0.01
0.00
Mean Harmonic Amplitude
Georges Bank 4yr
St.Pierre Bank 4yr
Bay of Fundy 4yr
Western Bank 4yr
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Harmonic Number

FIG. 3. Mean harmonic amplitude spectra for 4-yr-old scallops collected from four commercial scallop beds.

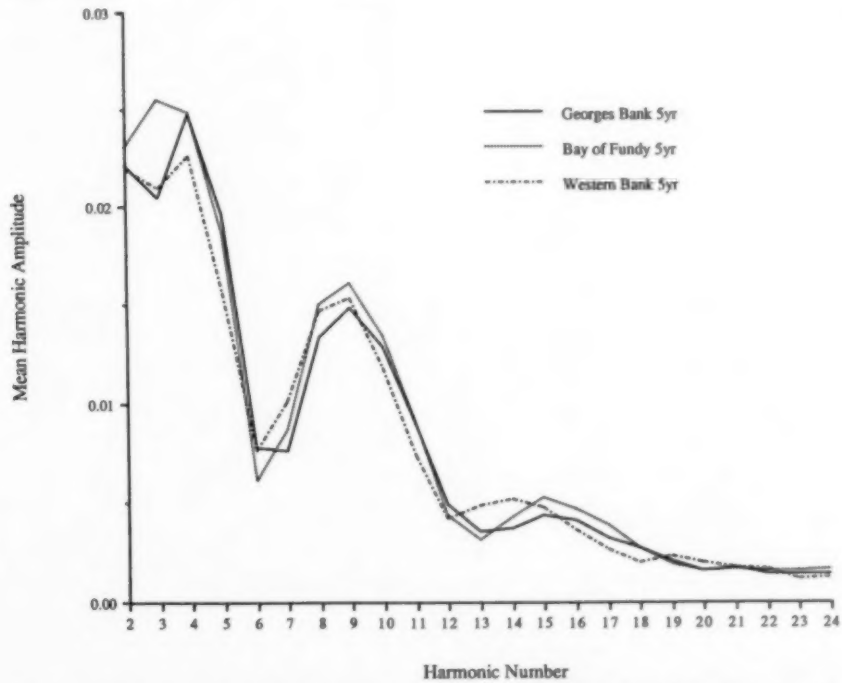
0.03
0.02
0.01
0.00
Mean Harmonic Amplitude
Georges Bank 5yr
Bay of Fundy 5yr
Western Bank 5yr
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Harmonic Number

FIG. 4. Mean harmonic amplitude spectra for 5-yr-old scallops collected from three commercial scallop beds in 1983.

sis, all of the harmonics except numbers 2, 15, 16, and 19–22 were significantly different among the samples. The correction for size had little impact on the analyses. The only change, if the size standardization was removed, was that harmonic 2 became significant. However, when samples representing only one year-class of 4-yr-old scallops were

TABLE 3. Results of post hoc analyses of harmonic amplitudes among beds indicating the relationships between the means for each harmonic number that showed a significant difference in ANOVA. Means outside of inequalities are not ordered according to magnitude.

Pattern of population means	Harmonic number
4-yr-old scallops	
St. Pierre Bank < Georges Bank = Western Bank = Bay of Fundy	5, 6, 10, 11, 12, 17
St. Pierre Bank > Georges Bank = Western Bank = Bay of Fundy	13, 14
St. Pierre Bank = Western Bank < Georges Bank = Bay of Fundy	9, 23, 24
Western Bank < Georges Bank = St. Pierre Bank = Bay of Fundy	7, 8
Georges Bank > Western Bank = St. Pierre Bank = Bay of Fundy	18
Bay of Fundy < Georges Bank = St. Pierre Bank = Western Bank	3
St. Pierre Bank = Bay of Fundy < Georges Bank = Western Bank	4
4-yr-old scallops captured in 1983	
Georges Bank = Western Bank < Bay of Fundy	10
Georges Bank = Western Bank > Bay of Fundy	3, 4, 18, 21
Georges Bank = Bay of Fundy > Western Bank	7, 8, 24
5-yr-old scallops captured in 1983	
Georges Bank = Western Bank < Bay of Fundy	8, 10, 15, 16, 17, 23, 24
Georges Bank = Western Bank > Bay of Fundy	2, 6, 12, 13, 19, 20
Georges Bank < Western Bank = Bay of Fundy	7, 9, 14, 22
Georges Bank > Western Bank = Bay of Fundy	5, 11, 18
Georges Bank = Bay of Fundy > Western Bank	4

analyzed (i.e., St. Pierre Bank excluded), only eight harmonic numbers were significantly different among beds. In contrast, the 5-yr-old scallops from the same year-class showed significant differences among beds in all harmonics except numbers 3 and 21 (Table 2). Correcting for size only altered the significance of harmonic 3 in that analysis.

ANOVA among ages within the same bed, with the same year of capture (i.e., different ages and year-classes), revealed significant shape differences in a variable number of harmonics depending on location (Table 2). When the same year-class was sampled in consecutive years on Western Bank, the harmonic numbers that define the hinge and auricles appear as significantly different. ANOVA of the two 5-yr-old year-classes sampled on Western Bank showed significant differences in most of the harmonics (Table 2).

Post hoc Analyses of Among-Bed Comparisons

Of the 16 significant ANOVAs of among-bed comparisons of 4-yr-old scallops not controlling for year-class, post hoc tests showed that eight were distinguishing between St. Pierre Bank scallops and those from other beds, while not distinguishing among the latter (Table 3). However, each bed was significantly different from each of the others in at least 3 of the 23 harmonics.

When 4-yr-olds from the same year-class were analyzed, two general patterns emerged. In those tests, five of the eight significant ANOVAs showed that the Bay of Fundy scallops had significantly different mean values from the offshore beds. The remaining analyses distinguished the scallops of Western Bank from those of Georges Bank and the Bay of Fundy.

Post hoc analyses of the bed mean amplitudes for 5-yr-old scallops also showed that the Bay of Fundy scallops were distinctive. Thirteen of the 21 analyses showed that the Bay of Fundy scallops had significantly different mean values from the offshore beds, while the latter sample means could not be distinguished from each other. In this age group, Georges Bank is significantly different from the other beds in seven harmonics, while Western Bank is significantly different from the others in harmonic 4.

A clinical arrangement of the magnitude of the means was not significant in any of the post hoc tests.

Discussion

Castonguay et al. (1991) evaluated the usefulness of Fourier analysis of otolith shape for discriminating between stocks of Atlantic mackerel (*Scomber scombrus*). They found that both age and year-class effects were significant. The resulting temporal instability in shape confounded their interest in using the harmonics as long-term stock descriptors. Age effects have also been reported in herring otolith shapes (Bird et al. 1986). In this study, both age and year-class effects have been shown to exert important influence on scallop shape harmonic amplitudes.

Allometric effects on Fourier harmonics also have a significant influence on shell shape in this species. *Placopecten magellanicus* has three life-history stages with respect to swimming characteristics (Dadswell and Weihs 1990). The first year of life is largely spent attached to the substrate by means of byssal threads. Then, at shell diameters of about 0.3 cm the scallops enter a mobile stage where swimming is common. In this stage, their shells have optimal hydrodynamic characteristics for swimming. After their shells reach a certain size and weight, generally over 10 cm in diameter, the animals enter the third stage during which they are largely sedentary.

Interpretation of the allometric shape differences observed in this study suggests that as the scallops enter the third-stage shell, the shape alters. The gross form becomes more circular, the hinge more prominent, and the auricles less well defined. Dadswell and Weihs (1990) observed that scallops of 10-cm shell diameter tended to have equal heights and widths, which is consistent with our observations, but that larger animals tended to be wider than they were long. This later trend during which the valve grows more in width than height results in the auricles being obscured. The function of the auricles is thought to relate to swimming (Dadswell and Weihs 1990) and so their decreased prominence with age is in accordance with a sedentary lifestyle. In the present study, life stage is a potential confounding effect in the data set. It would appear from their allometric relationships that the largest 5-yr-olds from Georges Bank, and Western Bank captured in 1983, have most likely entered the third, sedentary phase of their life history.

Comparisons among scallop beds separated by age and sampling year revealed significant differences in most harmonics. Each bed was significantly different from each of the others. However, two patterns become prominent when the harmonic analyses are considered as a whole. Amongst 4-yr-olds not selecting for year-class, the majority of the analyses found a significant difference between the St. Pierre Bank scallops and the others. This distinction of St. Pierre Bank may be due to the confounding effect of year-class, as this sample was collected in 1989 whereas the others were collected in 1983. However, there may also be a genetic basis for this pattern. Kenchington et al. (1993) reported differences in the number of common bands in the glutamic oxaloacetate transaminase (GOT) locus in St. Pierre Bank scallops from those reported for the Bay of Fundy and Scotian Shelf scallops (Gartner-Kepkay and Zouros 1985). These results suggest a degree of genetic isolation of St. Pierre Bank from the other beds in the study and imply a genetic component to the observed shape differences.

When only one year-class is analyzed, the Bay of Fundy scallop harmonics are significantly different from those of Georges and Western banks in most of the analyses. Amongst 4-yr-olds, this pattern is found in five of eight tests, and in 5-yr-olds, it is found in 13 of 21 tests. The Bay of Fundy is the only inshore bed of the three and the scallops there are under a strong tidal influence that may affect the shape of the shells.

The significant year-class effect on Western Bank suggests either that shell shape is influenced by environmental factors in early developmental stages, that it is influenced by the accumulated environmental effects experienced throughout the life span of the individual, or that the year-classes differ in average genotype, perhaps through larval supply from different areas. This year-class effect is particularly interesting given that it is stronger than the area differences between Western Bank and Georges Bank (Table 3).

In conclusion, Fourier analysis of the scallop shell shape has provided a sensitive tool for population analyses. The technique successfully distinguishes phenotypic differences even after controlling for allometric effects. Future studies of the population biology of the sea scallop should consider age, year-class, location, allometric, and life-stage effects in the experimental design. An exploration of the causes of the observed patterns including studying whether any of the shape differences have a genetic basis would be valuable.

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Application of a Generalized Logit Model to Condition Data for Trawl-Caught Pacific Halibut, *Hippoglossus stenolepis*

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Fisheries data commonly relate a categorical response variable to one or more continuous explanatory variables. We propose a generalized logit model for the analysis of such data. Our model differs from the standard generalized logit model in that transformation parameters for the explanatory variables are estimated simultaneously with other model parameters. We also employ likelihood inference and generic minimization techniques. Our model formulation leads to response surfaces with distinctly different shapes for different levels of the response variable. We illustrate our model with data on the condition of trawl-caught Pacific halibut, *Hippoglossus stenolepis*, as assessed by observers on commercial trawl vessels. In this data set, the probability of assigning a particular condition level to a fish depends significantly on the time the fish spent on the vessel's deck, fish length, total weight of the catch, tow depth, and tow duration.

Les données sur les pêches établissent couramment un lien entre une variable dépendante pour des catégories et une ou plusieurs variables indépendantes continues. Nous proposons un modèle logit généralisé pour l'analyse de ces données. Notre modèle diffère du modèle logit généralisé courant en ce sens que les paramètres de transformation des variables indépendantes sont estimés en même temps que d'autres paramètres du modèle. Nous utilisons également l'induction fondée sur la vraisemblance et la technique de la minimisation générique. La formulation de notre modèle produit des surfaces de réponse dotées de formes différentes distinctes pour différents niveaux de la variable dépendante. Notre modèle est illustré par des données sur l'état du flétan du Pacifique, *Hippoglossus stenolepis*, capturé au chalut, évalué par des observateurs à bord de chalutiers commerciaux. Dans cet ensemble de données, la probabilité d'attribuer un niveau d'état donné à un poisson dépend énormément du temps que le poisson a passé sur le pont du navire, de la longueur du poisson, du poids total des prises, de la profondeur du trait de chalut et de sa durée.

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Analysis of fisheries data often requires relating a response variable to one or more continuous explanatory variables. For example, Bilton et al. (1982) relate numbers of returning adult salmon to the time and size at release of hatchery juveniles. Schnute and McKinnell (1984) develop a general response surface analysis for interpreting such data. Response surface analysis is inappropriate, however, if the response is categorical (with k possible outcomes) rather than continuous. In this paper, we present methods that are analogous to response surface analysis and that are applicable to categorical response data. For example, our methods could be used to relate low, average, or high levels of fishery recruitment to spawning stock biomass and oceanographic conditions.

Methods for relating binary ($k = 2$) responses to continuous or discrete explanatory variables are well known (Cox and Snell 1989; Agresti 1990; Christensen 1990). The generalized linear model (McCullough and Nelder 1989) is suitable for many applications and the corresponding software is widely available. Loglinear and logit models are two special cases of the generalized linear model. Other methods have been developed in the fisheries literature for binary response data which are not adequately described by the generalized linear model. Schnute and Richards (1990) and Richards et al. (1990) present models that allow transformation parameters to be estimated simultaneously with other model parameters. By contrast, the generalized linear model requires the data to be transformed in advance.

Although methods are available to analyze categorical response data with $k > 2$, they are not in common use. Popular statistical software packages may not include options for this type of analysis, or may provide limited options only. Here, we present a model for relating categorical responses to one or more continuous explanatory variables, and we demonstrate the model's utility. Our method is based on the generalized logit model (Agresti 1990, p. 313). However, we modify the method to allow estimation of transformation parameters on the explanatory variables along with other model parameters. We employ standard methods of likelihood inference (Edwards 1972; Kendall and Stuart 1979) and generic minimization techniques for estimation.

Our application relates to the condition of trawl-caught Pacific halibut, *Hippoglossus stenolepis*. In British Columbia, Canada, halibut can be fished commercially by designated longline vessels only. Trawl vessels are required to discard all halibut in the catch as a condition of license. In a 1992 study to determine the mortality of trawl-caught halibut, observers on trawl vessels scored the condition of halibut

brought on board. They also measured fish size and recorded characteristics of the trawl tow and the length of time the halibut remained on deck before it was examined. Here, we estimate the probability that an observer will assign a particular condition score to a halibut, given its size, the amount of time it spent on deck, and the tow characteristics. These results are of interest to fishermen and fishery managers, who must find ways to minimize discard mortality in the trawl fishery.

1. Statistical Model

The goal of a generalized logit analysis is to predict the probability p_y of observing a particular response y, dependent on a vector $\mathbf{x} = (x_1, \ldots, x_m)$ of continuous explanatory variables. The response y is categorical, such that $y \in (1, \ldots, k)$. Thus, there are k probabilities $p_y(\mathbf{x})$ associated with each vector $\mathbf{x}$. The constraint

$$(1.1) \qquad \sum_{y=1}^{k} p_y(\mathbf{x}) = 1$$

implies that one of the probabilities $p_{y'}(\mathbf{x})$ is determined by the other $k - 1$ probabilities. The choice y' and the order of categories y are arbitrary. There may, however, be a biologically meaningful ranking for the categories y, as illustrated in our example below.

The generalized logit model proposed here is given by the three equations

$$(1.2) \qquad \xi_j(x_j) = \begin{cases} \dfrac{2x_j^{\alpha_j} - v_j^{\alpha_j} - u_j^{\alpha_j}}{v_j^{\alpha_j} - u_j^{\alpha_j}}, & \alpha_j \neq 0 \\ \dfrac{\log(x_j^2 / (v_j u_j))}{\log(v_j u_j)}, & \alpha_j = 0 \end{cases}$$

$$(1.3) \qquad \eta_y(\mathbf{x}) = \begin{cases} a_y + \sum_{j=1}^{m} b_{yj} \xi_j(x_j), & y \neq y' \\ 0, & y = y' \end{cases}$$

$$(1.4) \qquad p_y(\mathbf{x}) = \frac{\exp(\eta_y(\mathbf{x}))}{\sum_{z=1}^{k} \exp(\eta_z(\mathbf{x}))}$$

Equation (1.2) applies a modified power transformation (Schnute and McKinnell 1984) to each component of the data vector $\mathbf{x}$. This transformation accounts for the difference between our model and the standard generalized logit model (Agresti 1990). For each y, equation (1.3) then defines a linear predictor $(\eta_y(\mathbf{x})$ of the transformed data $\xi_j(x_j)$, dependent on the offset a_y and the coefficients $b_{y1}, \ldots, b_{ym}$. Equation (1.4) transforms the k predictors to probabilities p_y, such that the constraint (1.1) is satisfied.

The transformation (1.2) facilitates both parameter estimation and interpretation of the results. The exponents α_j $(j = 1, \ldots, m)$ in (1.2) act as transformation parameters, while the prescribed quantities $(u_j, v_j)_{j=1,\ldots,m}$ are selected by the user to represent typical low and high values, respectively, of x_j. These choices do not affect model structure; thus, different choices (u_j, v_j) merely induce parametric transformations on a_j and b_{yj}. Although other transformations, such as the Box–Cox transformation (Box and Cox 1964)

$$(1.5) \qquad \xi_j(x_j) = \begin{cases} \dfrac{x_j^{\alpha_j} - 1}{\alpha_j}, & \alpha_j \neq 0 \\ \log(x_j), & \alpha_j = 0, \end{cases}$$

could be used, the formulation (1.2) has the advantage that most transformed observations $\xi_j(x_j)$ lie within the range $(-1,1)$. Our experience has shown that the formulation (1.2) greatly reduces correlations among the parameter estimates $\hat{a}_y$ and $\hat{b}_{yj}$. Furthermore, search algorithms to locate maximum likelihood estimates tend to work more efficiently with (1.2) than with (1.5). Incidentally, in (1.2), we define $0^{\alpha_j} = 0$ when $\alpha_j > 0$. Thus, values $x_j = 0$ are allowed, provided that $\alpha_j > 0$.

The model (1.2)–(1.4) is essentially invariant to the choice y' in (1.3). Thus, for any $y' \in (1, \ldots, k)$, the same probabilities $p_y(\mathbf{x})$ in (1.4) can be achieved. In effect, different choices of y' correspond to different choices of the parameters a_y and b_{yj}. The quantity $\eta_y(\mathbf{x})$ in (1.3) describes a relative weight between level y and the standardized level y'. To simplify our discussion below, we choose $y' = k$.

Mathematically, the model expresses the probabilities p_y $(y = 1, \ldots, k)$ as functions of the vector $\mathbf{x}$ of exploratory variables. More precisely, the three model equations sequentially define $p_y(\mathbf{x}, \boldsymbol{\theta})$, where

$$(1.6) \qquad \boldsymbol{\theta} = (\alpha_1, \ldots, \alpha_m, a_1, \ldots, a_{k-1}, b_{11}, \ldots, b_{k-1,m})$$

is the vector of m transformation parameters α_j, $k - 1$ offsets a_y, and $(k - 1) \times m$ linear coefficients b_{yj}. A typical data set consists of pairs $(y_i, \mathbf{x}_i)$, where $\mathbf{x}_i = (x_{i1}, \ldots, x_{im})$ and $i = 1, \ldots, N$. The model's likelihood function is then the product of probabilities.

$$(1.7) \qquad L(\boldsymbol{\theta}) = \prod_{i=1}^{N} p_{y_i}(\mathbf{x}_i, \boldsymbol{\theta})$$

across all N observations. Estimates $\hat{\boldsymbol{\theta}}$ of $\boldsymbol{\theta}$ can be obtained by maximizing (1.7) or by minimizing the negative logarithm of $L(\boldsymbol{\theta})$. For convenience, we use twice the negative log likelihood

$$(1.8) \qquad \ell(\boldsymbol{\theta}) = -2 \sum_{i=1}^{N} \log(p_{y_i}(\mathbf{x}_i, \boldsymbol{\theta})),$$

which we term the "inference function".

Uncertainty in $\hat{\boldsymbol{\theta}}$ can be assessed from the estimated asymptotic covariance matrix

$$(1.9) \qquad \hat{V}[\hat{\boldsymbol{\theta}}] = \left[\frac{1}{2} \left(\frac{\partial^2 \ell(\hat{\boldsymbol{\theta}})}{\partial \theta_i \, \partial \theta_j} \right)_{i=1,\ldots,M;\, j=1,\ldots,M} \right]^{-1},$$

where M is the number of model parameters. The matrix (1.9) is derived from asymptotic properties of $\hat{\boldsymbol{\theta}}$ (Kendall and Stuart 1979, p. 59–60). Estimated standard errors $\widehat{\mathrm{SE}}[\hat{\theta}_i]$ for the components of $\hat{\boldsymbol{\theta}}$ are then given by $\sqrt{\hat{V}_{ii}}$, where $\hat{V}_{ii}$ is a diagonal component of (1.9).

Our analysis often involves testing the possibility that one or more of the factors x_j have statistical significance for the predicted probability p_y. Mathematically, this corresponds to a test of whether or not certain parameters b_{yj} can be constrained to 0. To compare two nested models, we use an asymptotic χ^2 likelihood ratio test. For example, suppose that models 1 and 2 have parameter vectors $\boldsymbol{\theta}_1$ and $\boldsymbol{\theta}_2$,

TABLE 1. Description of the levels used to code halibut condition (from Hoag 1975).

Level	Description
1	Vigorous body movement; minor external injuries
2	Weak body movement; minor external injuries
3	No body movement, but can tightly close operculum; minor external injuries
4	No body movement, but can move operculum; major injuries
5	No body or opercular movement

TABLE 2. Mean and standard error of each explanatory variable x_j at each level of the response y, corresponding to fish conditions from excellent ($y = 1$) to dead or moribund ($y = 5$). Sample sizes, sample proportions, and the means and standard errors for the entire data set are also given.

		Mean and standard error					
x	Description	$y = 1$	$y = 2$	$y = 3$	$y = 4$	$y = 5$	$y = 1, \ldots, 5$
x_1	Deck time (min)	8.6 ± 0.4	13.5 ± 0.5	19.8 ± 0.6	32.1 ± 1.1	70.4 ± 3.5	25.9 ± 0.8
x_2	Fish length (cm)	75.5 ± 0.9	75.0 ± 0.8	70.5 ± 1.0	71.0 ± 1.0	57.6 ± 1.4	70.7 ± 0.5
x_3	Catch weight (t)	2.20 ± 0.07	2.79 ± 0.08	3.10 ± 0.11	3.76 ± 0.11	3.70 ± 0.15	3.06 ± 0.05
x_4	Tow depth (m)	121 ± 3	148 ± 6	130 ± 5	182 ± 8	128 ± 10	142 ± 3
x_5	Tow time (h)	2.86 ± 0.05	2.92 ± 0.06	2.91 ± 0.06	3.27 ± 0.07	3.15 ± 0.08	3.00 ± 0.03
	Sample size	280	358	310	274	215	1439
	Sample proportion	0.19	0.25	0.22	0.19	0.15	1.0

respectively, where $\boldsymbol{\theta}_1$ is a constrained version of $\boldsymbol{\theta}_2$. We can reject model 1 in favor of model 2 with confidence level π if

$$(1.10) \quad \ell(\boldsymbol{\theta}_1) - \ell(\boldsymbol{\theta}_2) \geq \chi^2(\pi,d),$$

where d represents the number of constraints that model 1 imposes on model 2 and $\chi^2(\pi,d)$ denotes the π-level cutoff for χ^2 with d df. Thus, $d = M_2 - M_1$, where M_i denotes the number of free parameters in model i ($i = 1,2$).

The Akaike information criterion (Akaike 1974)

$$(1.11) \quad \text{AIC} = \ell(\hat{\boldsymbol{\theta}}) + 2M$$

provides a more informal means of model comparison, where M again denotes the number of free model parameters. A model with a low AIC value relative to other models is considered to provide a good model fit. The term $2M$ in (1.11) acts as a penalty for the number of parameters; a model is favored if it can achieve a low value of $\ell(\hat{\boldsymbol{\theta}})$ with few parameters. This test corresponds roughly to the formal test (1.10) because for $M > 1$, each additional df adds about two units to the χ^2 cutoff value.

2. Application

Data were collected from the bottom trawl fishery in Hecate Strait and off the west coast of Vancouver Island, British Columbia, between July and November 1992. Trained observers attempted to sample each halibut caught in a monitored tow. Sampled halibut were assessed for condition using criteria developed by Hoag (1975). According to Hoag's scale, level 1 represents a fish in excellent condition and a dead or moribund fish is scored as level 5 (Table 1). Complete data were available for 1439 fish, representing 98 tows on six trips.

Five possible explanators of fish condition were also recorded: (1) deck time; (2) fish length; (3) catch weight; (4) tow depth; and (5) tow time. Deck time was measured from when the net codend landed on the deck of the trawl vessel until the observer examined the fish. Fish length was recorded as total length. Tow weight was the estimated weight of the total catch from that tow. Tow depth was an estimate of the median depth fished if the bottom was uneven or sloped. Tow time was measured from when the net was in a fishing position on bottom until net retrieval was initiated. All halibut were caught using bottom trawl gear.

Deck time, catch weight, and tow time were positively related to fish condition (Table 2). Thus, not surprisingly, fish in the worst condition (level 5) had the longest mean deck time. They also tended to be caught in longer and heavier tows. Long and heavy tows may lead to a greater chance of injury through compaction or suffocation (Neilson et al. 1989). Fish length was negatively related to condition. Fish scored as level 5 had a mean length 18 cm smaller than fish scored as level 1, where most of the mean length reduction occurred between levels 4 and 5. There was no consistent trend between mean tow depth and fish condition.

Nonlinear techniques are required to obtain parameter estimates $\hat{\boldsymbol{\theta}}$ from the model (1.2)–(1.4) for the halibut data set. We used automatic differentiation (Griewank and Corliss 1991; Otter Research Ltd. 1992) in conjunction with a quasi-Newton search algorithm to minimize the inference function (1.8). We had occasional difficulty in locating a minimum with this technique because estimates $\hat{\alpha}_j$ would tend to $\pm\infty$. We opted to place arbitrary bounds of ± 3 on these exponents. The minimization procedure was well-behaved with these bounds in effect. Estimates $\hat{\alpha}_j$ were located away from the arbitrary bound in all but one of the cases we examined.

One advantage of automatic differentiation is a robust numerical scheme for calculating the covariance matrix (1.9). We used first differences of the automatic gradient to estimate the second derivatives. Our results were robust

TABLE 3. Estimates $\hat{\boldsymbol{\theta}}$ from the model (1.2)–(1.4), minima $\ell(\hat{\boldsymbol{\theta}})$ of the inference function, and corresponding AIC values. Ten cases represent the null model, five possible analyses with one explanatory variable, and the best two-, three-, four-, and five-variable combinations. Standard errors $\widehat{\mathrm{SE}}[\hat{\theta}_i]$ from (1.9) are also given for the five-variable model. In parameters α_y, a_y, and b_{yj}, the index y corresponds to the levels described in Table 1, and the index j corresponds to the variables x_j listed in Table 2. Selected values for the prescribed quantities (u_j, v_j) are (0 min, 100 min), (20 cm, 100 cm), (0.2 t, 8.0 t), (50 m, 450 m), and (1 h, 6 h), for $j = 1, \ldots, 5$, respectively. For case 6, $\hat{\alpha}_5 > 3$, but was constrained to $\hat{\alpha}_5 = 3$ by our arbitrary upper bound.

	Case										
$\hat{\boldsymbol{\theta}}$	1	2	3	4	5	6	7	8	9	10	SE
α_1	—	1.07	—	—	—	—	1.01	0.90	0.77	0.78	±0.13
α_2	—	—	−1.85	—	—	—	−2.29	−2.20	−1.84	−1.77	±0.42
α_3	—	—	—	2.07	—	—	—	2.68	2.67	2.68	±0.36
α_4	—	—	—	—	2.65	—	—	—	2.51	2.71	±0.47
α_5	—	—	—	—	—	3.00	—	—	—	2.47	±1.16
a_1	0.27	−8.09	−6.51	−2.17	−0.81	−0.34	−15.46	−17.96	−18.09	−18.69	±3.04
a_2	0.51	−3.91	−5.67	−0.24	0.43	0.15	−10.91	−11.09	−10.07	−10.42	±2.14
a_3	0.37	−1.74	−2.49	0.09	−0.09	−0.10	−4.05	−4.07	−4.64	−5.18	±1.05
a_4	0.24	−0.07	−2.91	0.22	0.38	0.34	−2.71	−2.72	−2.24	−2.61	±0.75
b_{11}	—	−13.45	—	—	—	—	−12.87	−12.03	−11.58	−11.97	±0.99
b_{12}	—	—	8.02	—	—	—	9.10	9.59	9.97	9.69	±2.40
b_{13}	—	—	—	−3.42	—	—	—	−4.28	−4.09	−4.84	±0.94
b_{14}	—	—	—	—	−1.30	—	—	—	−2.16	−2.10	±0.47
b_{15}	—	—	—	—	—	−0.93	—	—	—	−0.18	±0.59
b_{21}	—	−8.60	—	—	—	—	−8.41	−7.93	−7.70	−7.74	±0.50
b_{22}	—	—	7.36	—	—	—	8.25	8.46	7.89	8.04	±1.85
b_{23}	—	—	—	−1.24	—	—	—	−1.33	−1.35	−1.41	±0.27
b_{24}	—	—	—	—	−0.11	—	—	—	−0.95	−0.19	±0.39
b_{25}	—	—	—	—	—	−0.57	—	—	—	−1.50	±0.56
b_{31}	—	−5.34	—	—	—	—	−5.22	−5.05	−4.98	−5.05	±0.40
b_{32}	—	—	3.60	—	—	—	2.99	3.22	3.78	4.12	±0.79
b_{33}	—	—	—	−0.52	—	—	—	−0.52	−0.54	−0.63	±0.19
b_{34}	—	—	—	—	−0.59	—	—	—	−1.06	−0.38	±0.37
b_{35}	—	—	—	—	—	−0.73	—	—	—	−1.33	±0.52
b_{41}	—	−2.04	—	—	—	—	−1.98	−2.02	−2.09	−2.20	±0.31
b_{42}	—	—	3.94	—	—	—	3.18	3.25	2.99	3.24	±0.67
b_{43}	—	—	—	−0.06	—	—	—	−0.21	−0.21	−0.29	±0.13
b_{44}	—	—	—	—	0.22	—	—	—	−0.12	0.36	±0.28
b_{45}	—	—	—	—	—	0.17	—	—	—	−0.96	±0.40
$\ell(\hat{\boldsymbol{\theta}})$	4593	3536	4363	4428	4527	4553	3439	3342	3266	3232	
AIC	4601	3554	4381	4446	4545	4571	3467	3380	3314	3290	

to the choice of step size used for calculating the differences. The software available to us did not provide direct automatic calculation of second derivatives, although this is theoretically possible.

The first step in our analysis was to examine a "null" model in which fish condition is presumed independent of all five explanatory variables (Table 3, case 1). Mathematically, this corresponds to the constraints $b_{yj} = 0$ in (1.6), so that the model involves only the four parameters $a_1, \ldots, a_4$. (We have chosen $y' = 5$ in (1.3), as discussed in section 1.) The five quantities p_y calculated from the estimates $\hat{a}_1, \ldots, \hat{a}_4$ using (1.3)–(1.4) correspond precisely to the sample proportions in Table 2. Thus, in the absence of explanatory variables, the model conforms to the observed sample proportions. The minimum $\ell(\hat{\boldsymbol{\theta}})$ and the corresponding AIC for the null model serve as baselines against which to judge the significance of adding explanatory variables to the model.

We next applied the model individually to each of the five explanatory variables (Table 3, cases 2–6). As expected from the preliminary analysis (Table 2), deck time x_1 was the single most important variable; it was associated with the lowest AIC value. Other variables in order of importance were fish length x_2, catch weight x_3, tow depth x_4, and tow time x_5. AIC values corresponding to each model were substantially lower than the AIC value for the null model. Furthermore, based on the likelihood ratio test (1.10), the null model (case 1) can be rejected in favor of any univariate model (cases 2–6) with confidence level π greater than 99.9%.

Figure 1 illustrates the univariate relationships between the five explanatory variables and the predicted probabilities p_y for fish condition y. The deck time x_1 corresponding to the highest probability $p_y(x_1)$ increases with y (Fig. 1A). In particular, $p_1(x_1)$ is highest for short deck times, but rapidly drops to 0 as deck time increases. Conversely, $p_5(x_1)$ approaches 0 for short deck times and 1 for long deck times. This pattern is reversed for fish length x_2 (Fig. 1B). Thus, $p_5(x_2)$ approaches 1 for small fish, but decreases to 0 for large fish. For catch weight x_3 (Fig. 1C), the probabilities $p_4(x_3)$ and $p_5(x_3)$ increase with x_3; however, $p_1(x_3)$ tends to 0 as x_3 becomes large. In summary, a high probability of poor fish condition is associated with long deck times, the capture of small fish, or heavy tows. There is less contrast in the probabilities $p_y(x_4)$ and $p_y(x_5)$ for tow depth x_4 (Fig. 1D) and tow time x_5 (Fig. 1E), although the contrast increases with increasing x_4 or x_5.

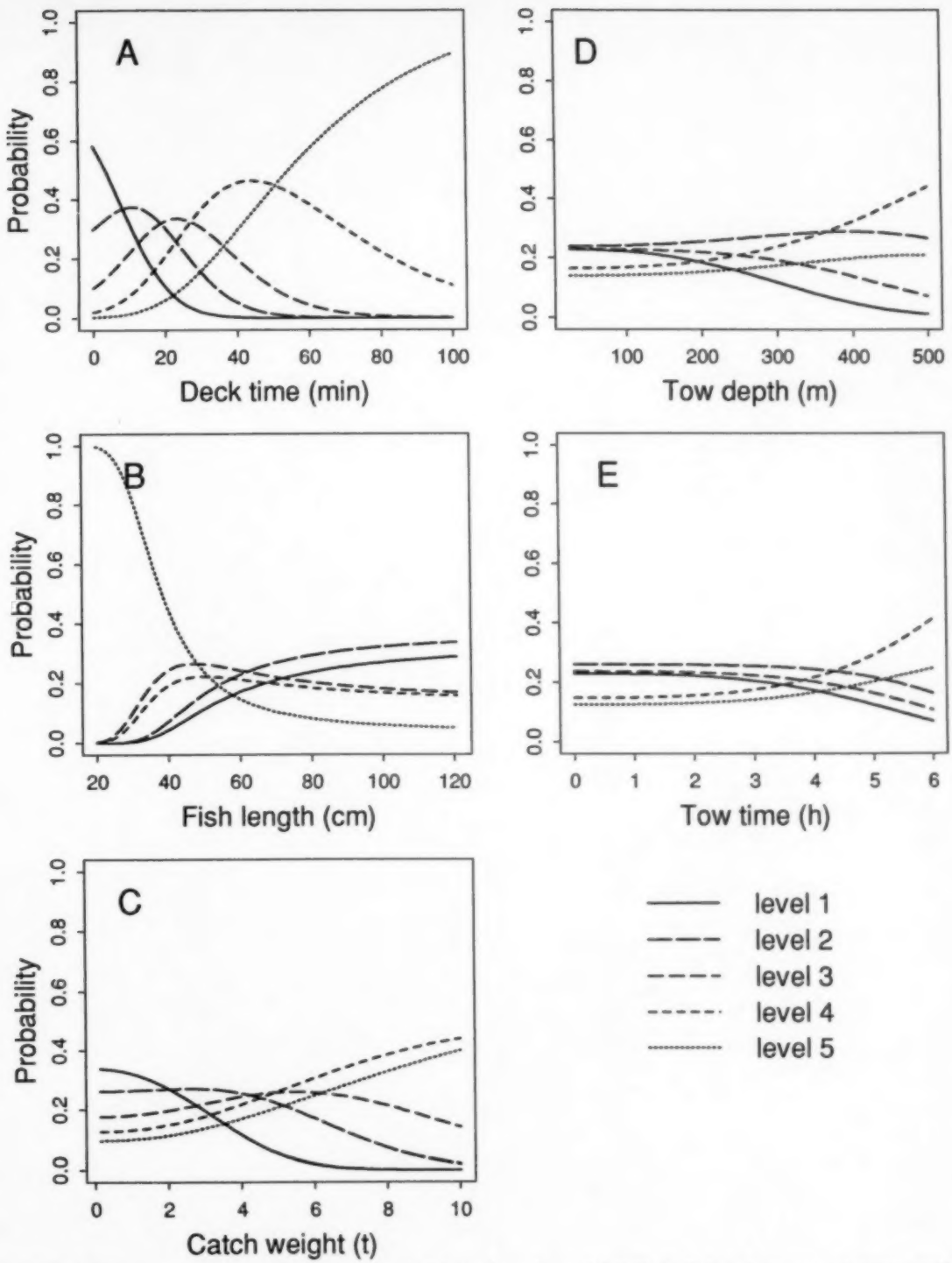

FIG. 1. Probabilities, $p_1, \ldots, p_5$ for each level of fish condition y from the model (1.2)–(1.4), based on one explanatory variable x_j. Panels A–E correspond to cases 2–6 (Table 3), respectively.

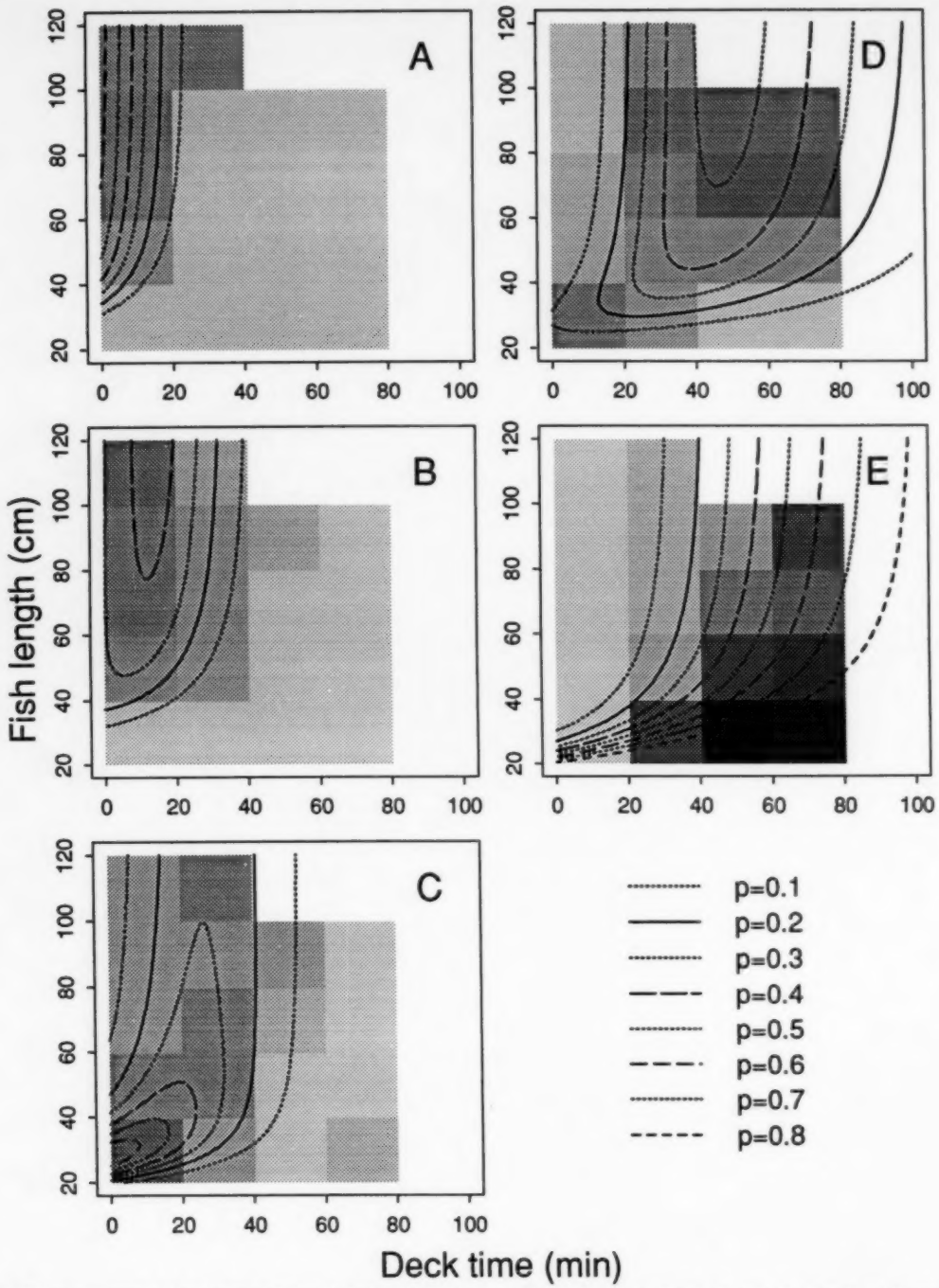

FIG. 2. Probability contours p_y for the model that includes deck time x_1 and fish length x_2 (Table 3, case 7). Panels A–E represent condition levels 1–5, respectively. Shaded (20 min × 20 cm) blocks indicate the percentage of observations (x_1,x_2) associated with fish condition y. Shading increases from the minimum to maximum value as the percentage of observations increases from 0 to 100%. Unshaded blocks represent regions with fewer than nine observations.

We next examined models with two explanatory variables. The model that included deck time x_1 and fish length x_2 (Table 3, case 7) had the lowest AIC. In two-dimensional space, the probabilities p_y can be examined as contours (Fig. 2). Shapes of these contours match the features apparent in Fig. 1A and 1B. Most of the contrast is in deck time and

the contours are largely independent of fish length for longer lengths. The shaded blocks in Fig. 2 indicate the percentage of observations (x_1,x_2) associated with fish condition y, where the shading increases from light to dark as the percentage increases from 0 to 100%. Unshaded blocks represent regions of sparse data. The two-variable model does appear to be a reasonable fit to the halibut data; the contours with highest probability pass through the darkest blocks in each case. For example, for y_3 (Fig. 2C) the darkest block represents the shortest deck time and smallest fish. Contour $p_3 = 0.6$ passes through this block only. Similarly, contour $p_4 = 0.2$ (Fig. 2D) bends to encompass a higher frequency in the bottom left block.

Models with three, four, and five explanatory variables lead to progressively lower values of the AIC (Table 3, cases 8–10). One interesting feature of these models is that the parameter estimates $\hat{\alpha}_j$, and $\hat{b}_{yj}$ tend to have similar values across all models that include those variables.

The five-variable model (Table 3, case 10) has an AIC value that is 24 units lower than the best four-variable model. Thus, by this criterion, the five-variable model is considered to be the "best fit" and all explanatory variables contribute significantly. Formal likelihood ratio tests based on (1.10) lead to the same conclusion. Not all the parameter estimates $\hat{b}_{yj}$ are significant; standard errors $\widehat{\mathrm{SE}}[\hat{\theta}_i]$ from (1.9) indicate that $\hat{b}_{15}$, $\hat{b}_{24}$, $\hat{b}_{34}$, and $\hat{b}_{44}$, do not differ significantly from 0. Thus, we could contemplate a reduced model with these parameters fixed at 0. However, such a model would be misleading because of the dependence of the estimates $\hat{b}_{yj}$ on the choice y'; a reduced model would not be invariant to an alternative choice of y'.

3. Concluding Remarks

The main contribution of our model (1.2)–(1.4) over the usual generalized logit formulation is the additional transformation (1.2). The importance of this transformation to our analysis is obvious from the standard errors in Table 3 (case 10); three of the five exponents $\hat{\alpha}_j$ differ significantly from 1. A more formal demonstration of the significance of the exponents can be conducted by comparing the final model (case 10) against an alternative model with the exponents α_j fixed at 1 (Table 4, case 11). Free exponents lead to a 97-unit reduction in the AIC value relative to the model with $\alpha_j = 1$. Thus, our use of (1.2) enables a substantially better fit to the overall model.

With fixed exponents α_j, the generalized logit model can be fit using commercially available software, for example, the SAS Institute Inc. (1989a) procedure CATMOD. One option for readers dependent on existing statistical software is to fix the transformation exponents α_j arbitrarily as a first step, then to estimate other model parameters, and finally to compare values of the AIC with different choices α_j. We obtained an AIC value reasonably close to the full model (Table 4, case 10) using fixed values α_j (case 12) chosen as integer approximations of the estimates $\hat{\alpha}_j$. Note that the AIC value generated from a commercial package should be adjusted to account for the extra loss in df from estimating the exponents α_j. Because of the combinatorial possibilities, comparisons among ad hoc choices become prohibitive with a large number m of explanatory variables. There are $7^5 = 16\,807$ possible comparisons for the halibut data if we consider only integer exponents in the range $[-3,3]$.

TABLE 4. Values of the minimum inference function $\ell(\hat{\boldsymbol{\theta}})$ and the AIC for different formulations of the generalized logit (GL) and proportional odds (PO) models, as determined by constraints on the exponents α_j and coefficients b_{yj} or b_j. The number of parameters M assumes that values of the exponents are estimated, unless $\alpha_j = 1$ for all j. Case 10 is repeated from Table 3 for comparison.

	Case				
	10	11	12	13	14
Model	GL	GL	GL	GL	PO
α_1	0.78	1	1	1	1
α_2	−1.77	1	−2	1	1
α_3	2.68	1	3	1	1
α_4	2.71	1	3	1	1
α_5	2.47	1	2	1	1
Offsets	a_y	a_y	a_y	a_y	—
Cutpoints	—	—	—	—	a_y
Coefficients	b_{yj}	b_{yj}	b_{yj}	b_y	b_y
M	29	24	29	9	9
$\ell(\hat{\boldsymbol{\theta}})$	3232	3339	3236	4047	3435
AIC	3290	3387	3294	4065	3453

Although our application has a natural order for the response levels, the model (1.2)–(1.4) does not acknowledge this order. Other possible formulations can be derived to exploit the ordering of response levels. For example, "proportional odds" models are based on cumulative probabilities

$$(3.1)\qquad q_y(\mathbf{x}) = \sum_{z=1}^{y} p_z(\mathbf{x})$$

that assume a natural ordering. The proportional odds model comparable with (1.2)–(1.4) is given by (1.2), the three equations

$$(3.2)\qquad \eta(\mathbf{x}) = \sum_{j=1}^{m} b_j \xi_j(x_j)$$

$$(3.3)\qquad q_y(\mathbf{x}) = \begin{cases} 0, & y = 0 \\ \dfrac{\exp(a_y + \eta(\mathbf{x}))}{1 + \exp(a_y + \eta(\mathbf{x}))}, & 1 \le y < k \\ 1, & y = k \end{cases}$$

$$(3.4)\qquad p_y(\mathbf{x}) = q_y(\mathbf{x}) - q_{y-1}(\mathbf{x}),\ 1 \le y \le k,$$

and the additional constraint

$$(3.5)\qquad a_1 \le a_2 \le \ldots \le a_{k-1}.$$

The constraint (3.5) guarantees that

$$(3.6)\qquad q_1(\mathbf{x}) \le q_2(\mathbf{x}) \le \ldots\ q_k(\mathbf{x}) = 1;$$

consequently, the probabilities $p_y(\mathbf{x})$ in (3.4) are positive and sum to 1. The quantities a_y in (3.3) are commonly termed cutpoint parameters.

Although the proportional odds model appears attractive, it contains a severe implicit restriction. The essential condition (3.6) on the functions $q_y(\mathbf{x})$ is achieved by the parametric constraint (3.5) on the cutpoints a_y. In general, (3.5) implies (3.6) only if the linear predictor $\eta(\mathbf{x})$ in (3.2)–(3.3) does not depend on y. Thus, the proportional odds (PO) model involves coefficients b_j in (3.2) that are independent of y. By contrast, the generalized logit (GL) model can incorporate

coefficients b_{yj} that depend on both the level y and the factor j, as in (1.3). The GL model can be restricted similarly to the PO model by imposing the constraint

$$(3.7) \qquad b_{yj} = b_j$$

in (1.3). Geometrically, the condition (3.7) leads to a GL model in which the functions $p_y(\mathbf{x})$, for every level y, all share the same contour surfaces where $\eta(\mathbf{x})$ is constant. In this case, only relative probability levels p_y attached to a specified contour differ among response levels y. Similarly, the PO model always contains this implicit geometric restriction.

In the context of response surface analysis, such a geometric restriction is quite natural; the continuous variate y depends on the factor vector $\mathbf{x}$, and each value of y defines a contour in factor space. The PO model mimics response surface analysis by associating ranked levels y with cumulative probabilities q_y; in effect, y is replaced by a variate in the range [0,1]. Figure 2 illustrates why the geometric limitations of the PO model may not always be adequate for categorical data. Distinct levels y can define distinct sets of probability contours; for example, contours in Fig. 2C have shapes different from those in Fig. 2D. Thus, the PO model appears too restrictive geometrically for the halibut data.

The name "proportional odds" derives from the property

$$(3.8) \qquad \frac{q_y(\mathbf{x})}{1-q_y(\mathbf{x})} = \frac{q_z(\mathbf{x})}{1-q_z(\mathbf{x})} \exp(a_y - a_z),$$

that follows mathematically from (3.3)–(3.4), where y and z are any two response levels. The cumulative odds ratios (3.8) are proportional to each other, with constants of proportionality independent of the factor vector $\mathbf{x}$. Similarly, the GL model restricted by (3.7) has the property

$$(3.9) \qquad p_y(\mathbf{x}) = p_z(\mathbf{x}) \exp(a_y - a_z),$$

so that probabilities $p_y(\mathbf{x})$, rather than odds ratios $q_y(\mathbf{x})/(1 - q_y(\mathbf{x}))$, have proportionality constants independent of $\mathbf{x}$.

Cases 13 and 14 (Table 4) report AIC values for the GL model restricted by (3.7) and the PO model, respectively. We made the simplifying assumption that $\alpha_j = 1$ for all j, allowing us to obtain the PO solution from the SAS Institute Inc. (1989b) procedure LOGISTIC. Thus, both cases 13 and 14 would lead to linear contour plots. The PO model resulted in a substantially lower AIC value than the restricted GL model. We originally considered analyzing the halibut data with a PO model. However, that model was rejected on the basis of a score test of the common contours assumption provided in the SAS output. A similar conclusion is evident from Table 4; the PO model (case 14) leads to a much higher AIC value than the GL model without the constraint (3.7) on the coefficients b_{yj} (case 11).

We have presumed that readers can access general minimization methods and construct the appropriate computer code for the model (1.2)–(1.4). Readers dependent on existing software packages may be forced to make ad hoc choices α_j, such as $\alpha_j = 1$ for all j. We know of no commercial package that currently accommodates simultaneous estimation of transformation parameters. Agresti (1990, Appendix A) reviews commercial software that provides routines for standard generalized logit and proportional odds analyses. Our philosophy is to place generalized logit analysis within the broader framework of likelihood and estimation methods commonly applied in fisheries modeling.

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Effects of Rearing Density on the Growth Response of Juvenile Fathead Minnow (*Pimephales promelas*) under Toxicant-Induced Stress

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The effects of rearing density on the apparent toxicity of pentachlorophenol and 2,4,5-trichlorophenol were investigated using 28-d growth and survival tests with larval fathead minnow (*Pimephales promelas*). The fish were held at rearing densities of 1, 5, or 10 fish per 1-L cage. Among individual fish, those held at the lowest density showed three times less variability in wet weight at 28 d than those at the highest density. Two-way analysis of variance of the growth data showed a significant interaction between concentration and density for both toxicants, suggesting that density affected the nature of the relationship between exposure concentration and response. The change in the concentration–response relationship was most dramatic for 2,4,5-trichlorophenol; a quadratic function for fish reared at low and medium densities changed to a linear function at the highest density. The estimated thresholds of toxicant impact on growth were twofold lower for low- versus high-density fish for both chemicals. Testwater volumes and replenishment were high enough in these experiments that decreased toxicant loading at higher densities did not appear to be a factor contributing to these results.

Les effets de la densité d'élevage sur la toxicité apparente du pentachlorophénol et du 2,4,5-trichlorophénol ont été examinés par le biais d'études de croissance et de survie de 28 jours à l'aide de larves de tête-de-boule (*Pimephales promelas*). Les poissons étaient placés à raison de 1, 5 et 10 poissons par cage de 1 L. Dans le cas de poissons placés seuls dans une cage, c'est-à-dire à la plus faible densité, le poids frais à 28 jours était trois fois moins variable que chez ceux élevés à la plus forte densité. L'analyse de la variance à deux facteurs des données de croissance a révélé une interaction importante entre la concentration et la densité des deux produits toxiques, ce qui laisse entendre que la densité affectait la nature du rapport entre la concentration d'exposition et la réaction. La modification du rapport concentration–réaction était plus marquée dans le cas du 2,4,5-trichlorophénol; une fonction quadratique pour les poissons élevés à faible et moyenne densité se transformait en une fonction linéaire pour la densité la plus élevée. Les seuils estimés de l'effet des produits toxiques sur la croissance était deux fois plus faibles pour les poissons élevés à faible densité par rapport à ceux élevés à haute densité pour les deux produits chimiques. Les volumes d'eau à analyser et l'alimentation en eau étaient suffisamment élevés dans ces expériences qu'une diminution de la charge en produit toxique à densité élevée ne semblait pas être un facteur contribuant à ces résultats.

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Growth is a common endpoint used to study the toxicity of both pure chemicals and complex effluents to aquatic organisms including fish. Growth is an important physiological parameter with obvious implications at the population level. Scope for growth is considered to be a nonspecific indicator responsive to any kind of stress, either natural or anthropogenic, which can be applied in both field-monitoring and laboratory-assessment schemes (Geisy and Graney 1989). Any toxicant stress will result in energy expenditure, with a concurrent reallocation of energy away from growth or reproduction. In controlled energetic studies, toxicants reduce energy conversion efficiency and growth in fish (Webb and Brett 1973; Borgmann and Ralph 1986). Field studies have demonstrated reduced growth as a sign of toxicant stress in fish populations in the field (Munkittrick and Dixon 1989).

In Canada and the United States, standardized growth and survival tests have been developed for use in studies to establish water quality criteria, as well as to assess the toxicity of industrial effluents (CCREM 1987; USEPA 1989; Environment Canada 1992). The growth response in these tests has, however, been criticized for its lack of sensitivity relative to other endpoints including even mortality (Woltering 1984). One problem in interpreting results of growth under toxicant stress may be related to density-dependent competition for food and space. Density-related stress has behavioural effects on fish that may lead to increased variation in the growth of individuals (Brown 1957; Ricker 1958; Jobling 1983). The extent to which density can interact with toxicant-related effects has not, however, been adequately examined.

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This study was undertaken to determine if something seemingly as simple as rearing density could alter the growth response of fathead minnow (*Pimephales promelas*) exposed to toxicant stress. Early life stage growth and survival studies were completed at different fish densities with two common organic contaminants known to have different modes of toxic action: pentachlorophenol (PCP), a metabolic uncoupler (Weinbach 1957), and 2,4,5-trichlorophenol (TCP), a polar narcotic (Kobayashi et al. 1979).

Materials and Methods

A factorial design was used to determine the effect of rearing density on the toxicity of PCP and TCP to larval fathead minnow. One-day-old minnow were exposed to nine levels of either PCP (nominally 0, 15, 30, 45, 60, 75, 90, 120, and 150 $\mu g \cdot L^{-1}$) or TCP (nominally 0, 75, 150, 225, 300, 450, 540, 720, and 900 $\mu g \cdot L^{-1}$). These sublethal toxicant levels were chosen on the basis of preliminary range-findings assays. The fish were held at one of three rearing densities: low (LO, 1 fish per 1-L cage), medium (MD, 5 fish per 1-L cage), or high (HI, 10 fish per 1-L cage). The toxicant concentration and density treatments for each experiment were carried out in triplicate, while the toxicant-exposure control was repeated six times, for a total of 90 groups: (8 toxicant concentrations × 3 rearing densities × 3 replicates) + (1 control × 3 rearing densities × 6 replicates). Two control replicates were eliminated from the PCP study due to the limited availability of fish at the start of the test; only four control replicates were included. Treatment effects and interactions were assessed on the basis of fish survival over 28 d and the wet weight of surviving fish at 28 d.

Larval fathead minnow were from a culture maintained at the University of Waterloo. Routine maintenance practices were based on those of Denny (1987). The culture system was operated at 23–25°C, with all tanks receiving a continuous flow of aerated groundwater. A photoperiod of 16 h light: 8 h dark, with artificial 15-min dawn and dusk periods, was employed for both culturing and subsequent experimentation.

Each toxicity test used a 30-tank exposure system. Each 20-L glass tank (aquarium), with volume adjusted to 13 L by an internal standpipe, received a continuous flow (100 $mL \cdot min^{-1}$) of toxicant solution or control water from a peristatic pump and diluter system. Eight of the 10 pump channels (two were controls) delivered stock solution to a chamber where it was mixed with dilution water. The mixture then passed to a three-way flow splitter, and thence to three replicate tanks. The tanks were aerated, largely to enhance circulation, by placing an airstone in a inclined glass tube attached to the inside length of each tank with non-reactive silicone adhesive. Nine removable cylindrical cages were placed in each tank to allow fish to be held at different rearing densities within the same exposure tank. The cages (effective volume when in use, 1000 cm^3) were constructed by attaching a 15-cm-high cylinder of 450-μm-mesh polypropylene screening to a glass petri dish bottom (diameter, 10 cm; height, 1.5 cm).

Separate stock solutions of PCP (purity >99%, Sigma Chemical Co., St. Louis, Mo.) and TCP (purity >98%, Aldrich Chemical Co., Milwaukee, Wis.) were used for each exposure concentration. The solutions were prepared with double-distilled water and contained a molar excess of NaOH to form the soluble salt of each phenol. The dilution water for the studies came from the normal groundwater supply to our laboratory; a full characterization is given in Kevan and Dixon (1991). During the studies, water temperature, pH, and dissolved oxygen were determined daily in randomly selected tanks, while total hardness (EDTA titration; APHA 1980) and alkalinity (HCl titration; APHA 1980) were measured periodically in randomly selected tanks. The mean ± SE, (n) total hardness, alkalinity, and pH were 392 ± 8 (14) $mg \cdot L^{-1}$ as $CaCO_3$, 278 ± 5 (14) $mg \cdot L^{-1}$ as $CaCO_3$, and 7.8 ± 0.1 (56), respectively. Water temperature ranged between 23 and 25°C, while dissolved oxygen was consistently >80% saturation.

Toxicant flow rates in the diluter system were monitored daily, and water samples for the determination of PCP or TCP concentration were drawn from a minimum of two exposure tanks each day. The samples (PCP, 400 mL; TCP, 100 mL) were taken such that each concentration was sampled at least twice a week and all tanks were sampled at least twice during each experiment. Each water sample was acidified with 10 mL of 0.5 M HCl and extracted into 10 mL of hexane. The hexane extracts were stored for a maximum of 1 wk at 4°C in teflon-capped amber-glass vials pending analysis. Concentrations of the phenolics were determined with a Spectra Physics SP8100 high-performance liquid chromatograph (HPLC) with a SP8400 variable wavelength UV detector set at 235 nm. The HPLC was equipped with a 50-μL sample loop and a C-8 reverse-phase column (length, 15 cm; inside diameter, 4.6 mm; particle size, 5 μm; Supelco Canada Ltd., Oakville, Ont.). The hexane extracts were back extracted into 10 mL of 0.2 M NaOH prior to analysis. TCP and PCP were eluted isocratically with a methanol–water (75:25) mixture containing 1% (v:v) acetic acid at a flow rate of 1 $mL \cdot min^{-1}$. The retention times for PCP and TCP were, respectively, from 7 to 8 and from 3 to 4 min. TCP was used as the internal standard for PCP determination, while PCP was used as the internal standard for TCP analysis. The method gave recoveries between 97 and 102% for both PCP and TCP. All results are expressed in terms of assayed phenolic concentration not corrected for recovery.

Each experiment followed a protocol similar to that for the USEPA (1989) 7-d fathead minnow growth and survival test. The test duration was, however, extended to 28 d, and deviations from standard protocol were incorporated in order to test specific hypotheses about density-related effects on growth. The tests were initiated by randomly transferring <24-h-old larvae to the polypropylene-screen cages and placing them in the exposure tanks. Each tank contained two HI-density cages, two MD-density cages, and five LO-density cages. Larvae that died during the first 24 h of the study were replaced with larvae of similar age; such mortality, attributed to initial handling stress, was minimal.

Feeding was quantified on a per-fish basis and standardized between cages by feeding set volumes of a slurry of concentrated live brine shrimp (*Artemia salina*) in water (Table 1) as suggestd by Neville (1989). Nauplii were collected by straining the saltwater culture solution through a brine net and then rinsing with freshwater. The nauplii were resuspended in freshwater to produce a standard feeding concentration. A repeater pipette with a modified tip was used to dispense equal volumes of the concentrate to each cage on a per-fish basis. Thus, while the concentration of the shrimp solution may have varied slightly from feeding to

TABLE 1. Food and feeding rates used during the 28-d PCP and TCP toxicity studies with larval fathead minnow. The fish were fed three times a day with a live brine shrimp slurry.

Study	Fish age (d)	Brine shrimp age (h)	Volume of slurry fed ($\mu L \cdot fish^{-1} \cdot feeding^{-1}$)	Estimated dry weight of feed	
				$mg \cdot fish^{-1} \cdot feeding^{-1}$	$mg \cdot fish^{-1} \cdot d^{-1}$
PCP	1–7	<12	10	0.4	1.2
	8–20	24	10	0.5	1.5
	21–27	24	20	0.8	2.4
TCP	1–6	<12	20	0.8	2.4
	7–15	24	20	1.0	3.0
	16–23	24	30	1.2	3.6
	24–27	24	40	1.6	4.8

TABLE 2. Survival and final wet weights of larval fathead minnow exposed to waterborne PCP for 28-d posthatch. The fish were held at LO (1 fish per 1-L cage), MD (5 fish per 1-L cage), or HI (10 fish per 1-L cage) rearing densities. Values are given as means ± SD (n). Within columns for survival and weight, an asterisk denotes a significant difference ($p \leq 0.05$) from controls.

Measured water concentration ($\mu g \cdot L^{-1}$)	Pooled survival (%)[a]	Wet weight (mg)[b]		
		LO	MD	HI
<1–(10)	98.3 ± 2.9	42.6 ± 6.2 (20)	40.6 ± 12.8 (40)	32.4 ± 12.9 (76)
14 ± 2 (15)	98.3 ± 1.7	42.0 ± 4.9 (15)	37.7 ± 12.0 (29)	33.5 ± 11.4 (59)
29 ± 5 (15)	100 ± 0.0	38.8 ± 4.9 (15)	37.5 ± 10.7 (30)	32.3 ± 12.6 (60)
39 ± 3 (16)	94.1 ± 8.2	38.5 ± 5.5 (13)	37.2 ± 9.6 (30)	30.7 ± 11.1 (53)
56 ± 6 (24)	98.9 ± 1.9	40.1 ± 7.0 (15)	34.9 ± 11.4 (30)*	27.2 ± 13.7 (58)
71 ± 4 (15)	91.7 ± 6.0	33.9 ± 6.7 (14)*	37.2 ± 12.0 (29)	31.4 ± 13.5 (51)
85 ± 11 (10)	91.6 ± 2.9	35.0 ± 4.8 (14)*	38.7 ± 10.0 (28)	26.0 ± 11.6 (53)
110 ± 21 (10)	85.0 ± 10.2	28.2 ± 5.5 (12)*	31.6 ± 9.7 (29)*	26.3 ± 14.1 (47)
133 ± 17 (24)	88.3 ± 4.4	26.9 ± 5.7 (14)*	31.9 ± 9.4 (26)*	24.1 ± 12.5 (51)*

[a]Since there was no significant impact of density on survival, the results of the density treatments were pooled to give three replicates for each concentration (four for controls).

[b]Means and SDs presented are based on all fish within each treatment. The statistical differences from controls, however, were determined using mean replicate weights (n = 3 for exposure treatments, n = 4 for controls).

feeding, the spatial variation in food offered at each feeding (among cages on a per-fish basis) was maintained with a reasonable degree of accuracy. The fish were fed three times a day at the levels indicated in Table 1. Attempts were made to roughly quantify the dry weight of food offered. Aliquots of the food concentrate were lyophilized and weighed to determine dry weight (Table 1).

Mortality was checked daily throughout the study; the number of fish surviving in each cage was recorded daily and at the end of the experiment. Based on mortality, the quantity of food offered was adjusted daily to maintain constant feeding rates. At the end of the 28-d exposure period, the fish were collected from the cages, killed in ice water, blotted dry on paper towelling, and weighed to the nearest 0.1 mg.

The coefficient of variation (CV) in mean wet weight at 28 d was calculated for the control fish at each rearing density as CV in % = [(100) (standard deviation of mean weight)] ÷ [mean weight]. Prior to testing for effects, all data sets were checked for homogeneity of variance and normality using Bartlett's F-test and probability plots. Survival data was expressed as a proportion of the original number of larvae in each treatment and transformed (arcsine square root) for variance stabilization. Because preliminary analysis of variance (ANOVA) showed no effect of density on survival, the data for the cages within each tank were pooled to give three replicate measures (four and six replicates for PCP and TCP controls, respectively) for each exposure concentration. Concentration effects on survival were then assessed by one-way ANOVA followed by Tukey's comparisons of means.

The wet weight data were normally distributed and the variance was homogeneous. Two-way ANOVAs were performed to test for any interactive effects of toxicant concentration and rearing density on wet weight. Regression analyses (both linear and quadratic) were also undertaken to further delineate the nature of the concentration–response relationships. The no-observed effect concentration (NOEC), the lowest-observed effect concentration (LOEC), and the threshold effect value (the geometric mean of the LOEC and NOEC) were determined for growth for each density level. The between-tank (among-replicate) variation was employed as the appropriate error term for all tests of significance. Significant differences among treatments within density groups were determined using ANOVA followed by Tukey's multiple comparison test. The regression analyses were completed using the Statistical Analysis System (SAS 1982). All other analyses used personal computer based SYSTAT 4.2 (Wilkinson 1988). The significance of all tests was determined at $\alpha = 0.05$.

TABLE 3. Survival and final wet weights of larval fathead minnow exposed to waterborne TCP for 28-d posthatch. The fish were held at LO (1 fish per 1-L cage), MD (5 fish per 1-L cage), or HI (10 fish per 1-L cage) rearing densities. Values are given as means ± SD (n). Within columns for survival and weight, an asterisk denotes a significant difference ($p \leq 0.05$) from controls.

Measured water concentration ($\mu g \cdot L^{-1}$)	Survival (%)[a]	Wet weight (mg)[b]		
		LO	MD	HI
<1–(8)	94.2 ± 3.6	59.9 ± 7.9 (27)	58.5 ± 20.1 (58)	50.5 ± 19.5 (115)
68 ± 4 (8)	91.1 ± 3.8	59.9 ± 6.1 (14)	55.5 ± 17.6 (28)	54.1 ± 14.0 (52)
130 ± 18 (8)	91.7 ± 4.4	61.6 ± 8.7 (14)	56.5 ± 16.3 (26)	49.8 ± 12.8 (57)
187 ± 15 (8)	96.1 ± 6.8	60.9 ± 8.4 (15)	49.0 ± 18.3 (30)	49.1 ± 17.3 (53)
297 ± 17 (8)	85.0 ± 2.9	56.2 ± 11.8 (13)	52.1 ± 15.8 (26)	40.7 ± 19.1 (49)*
398 ± 22 (8)	99.0 ± 1.9	46.5 ± 12.1 (15)*	55.4 ± 13.4 (29)	48.4 ± 15.6 (60)
536 ± 50 (8)	89.4 ± 8.2	46.5 ± 10.9 (14)*	55.3 ± 19.0 (24)	48.7 ± 19.9 (57)
725 ± 23 (8)	90.5 ± 4.8	32.7 ± 9.2 (14)*	42.3 ± 15.7 (28)*	40.1 ± 16.4 (51)*
863 ± 34 (8)	82.8 ± 7.8	27.3 ± 8.0 (12)*	24.4 ± 13.1 (23)*	41.5 ± 15.9 (55)*

[a]Since there was no significant impact of density on survival, the results of the density treatments were pooled to give three replicates for each concentration (six for controls).

[b]Means and SDs presented are based on all fish within each treatment. The statistical differences from controls, however, were determined using mean replicate weights (n = 3 for exposure treatments, n = 6 for controls).

Results

Preliminary analyses indicated that there were no significant effects of rearing density on the survival of fathead minnow at any PCP or TCP concentration tested. Low overall mortality and lack of variation within some treatments, however, limited the power of the statistical methods used to assess density effects, as well as concentration by density interactions, on survival. Complete survival data, by density and concentration, are available in Arthur (1991). Based on the above, the survival data were pooled with respect to density and reanalyzed to assess concentration effects. There were no significant concentration effects on larval survival in either study: mean survival exceeded 80% for all concentrations of PCP and TCP (Tables 2 and 3).

Rearing density had a marked effect on the variability of growth of individual fish even without exposure to a toxicant. For control fish in both experiments, the CV for growth increased dramatically as density increased from LO to HI (CV = 14.5 and 13.2%, 31.5 and 34.4%, and 39.8 and 38.6% for PCP and TCP control fish at LO, MD, and HI densities, respectively). Similar density-related effects on variability in growth of fish were observed at all toxicant exposure levels for PCP and TCP (Tables 2 and 3). In general, the LO-density cages showed less variation in fish weight within tanks than between tanks. In both the MD and HI treatments, the variance in fish weights within tanks and cages were greater than the variance between tanks. For both studies, the mean weight of control fish at 28 d was inversely related to density; mean weight increased as density decreased.

To determine if there were significant interactions between the effects of toxicant concentration and density on fish growth, two-way ANOVAs were completed for both studies using the mean fish weights for each replicate. Interactions were apparent for both PCP ($p = 0.002$) and TCP ($p < 0.0001$). The nature of the interaction effect can be seen more clearly from the results of the regression analyses. For PCP (Fig. 1), toxicant-induced effects on growth were greatest for LO-density fish independent of changes in within-treatment growth variation. The same effects was observed for TCP (Fig. 2), where the shape of the concentration–response curves also changed significantly between density treatments. The data for the LO- (Fig. 2A) and MD-density fish (Fig. 2B) were better described by a quadratic model than a linear model. In both cases, analysis of model residuals indicated that the concentration–response curve had a "breakpoint" rather than a smooth curve that would be expected of a true quadratic relationship. This breakpoint may be indicative of the threshold for TCP effects on growth: the breakpoint occurred at a significantly lower TCP concentration for the LO-density fish (between 187 and 297 $\mu g \cdot L^{-1}$) relative to the MD-density fish (between 536 and 725 $\mu g \cdot L^{-1}$). Interestingly, the concentration–response curve for HI-density fish was best described by a linear relationship.

Due to this density effect, threshold values varied considerably based on the density at which fish were held during the experiment. At the LO density, the lowest exposure concentration showing a statistically significant difference in wet weight relative to control fish (LOEC) was 71 μg PCP$\cdot L^{-1}$, with effects increasing in a concentration-dependent manner above the LOEC. The calculated threshold concentration for PC impact on growth was 63 $\mu g \cdot L^{-1}$. Results were less consistent for the MD-density fish. While the LOEC occurred at 56 μg PCP$\cdot L^{-1}$, higher concentrations showed no effect until 110 $\mu g \cdot L^{-1}$; lack of a suitable exposure–response relationship precluded calculation of a threshold. For HI-density fish, the LOEC was 133 μg PCP$\cdot L^{-1}$, with a threshold of 121 $\mu g \cdot L^{-1}$, approximately twice the threshold apparent with LO-density fish.

The final weights attained by the control fish during the TCP study (Table 3) were substantially higher than those attained by controls during the PCP study, presumably due to the higher feeding rate used during the former. The impacts of density on interpretation of the toxic effects of TCP paralleled those evident for PCP. The LOEC and threshold concentrations were, respectively, 398 and 344 μg TCP$\cdot L^{-1}$ for the LO-density fish and 725 and 623 μg TCP$\cdot L^{-1}$ for the MD-density fish. The reduced mean growth for HI-density fish at 297 $\mu g \cdot L^{-1}$ was the result of very poor growth (with

no apparent explanation) in one of the three replicates. When the result at 297 $\mu g \cdot L^{-1}$ is considered to be an outlier and is disregarded, the LOEC and threshold for the HI-density treatment are the same as those for MD density.

Discussion

Density-dependent competition for food and space has been shown to cause aggressive behaviour in some species of fish leading to variation in the growth of individuals. This phenomenon has been termed the size-hierarchy effect (Brown 1957) or growth depensation (Ricker 1958). Jobling (1983) found a size-hierarchy effect with Arctic char (*Salvelinus alpinus*) raised at different densities. Char reared in individual containers had a smaller CV for growth than fish raised together. Increased variation in growth was attributed to the effects of hierarchical behaviour where dominant (larger) fish maintained normal growth, while subdominant (smaller) fish grew more slowly, even though there was an excess of food. The effects on Arctic char growth were increased when fish were fed less often, although still to satiation at each feeding. Halver (1989) described studies that suggested that disproportionate food intake between larger and smaller fish, and hence differences in growth rate, could largely be attributed to differences in activity, food intake, and a generalized stress response. Peters (1983) suggested a possible physiological mechanism, although probably a secondary one, for hierarchical effects observed in studies with American eel (*Anguilla rostrata*); when eels were placed in a tank with a dominant individual, their stomachs became essentially nonfunctional.

A size-hierarchy effect was obvious in the present study; the CV for growth was reduced by approximately threefold in fish reared in the LO-relative to the HI-density treatments. LO-density fish showed less variation in fish weight within tanks than between tanks, probably due to small differences between tanks in either toxicant concentration or environmental variables such as temperature or light intensity. For both the MD and HI fish, the variation in final weights within tanks, and within cages, was greater than the variance between tanks. In these fish, the tank effect was masked by the density effect observed within the individual rearing cages. This size-hierarchy effect was not visibly apparent in terms of aggressive behaviour among fish; however, it was most likely due to density-dependent competition for space and/or food.

Examination of the literature suggests that variability in fathead minnow growth during other toxicity tests where the fish were reared together is comparable with the variation noted here for MD- and HI-density fish. Benoit et al. (1982), Holcombe et al. (1982), and Spehar et al. (1985) all reported within-treatment CVs of from 30 to 40%. Studies with flagfish (*Jordanella floridae*) have resulted in CVs for growth of control fish as high as 85% (Smith et al. 1991). The artificial nature of exposure chambers has also been shown to affect results in acute studies. In studies with zinc, hierarchical behaviour among bluegill (*Lepomis macrochirus*) resulted in the increased survival of dominant fish relative to submissive fish (Sparks et al. 1972). Incorporation of environmental heterogeneity into the test chamber, by placing something as simple as a clay flowerpot in the tanks, was sufficient to diminish the effect.

Woltering (1984) and Suter et al. (1987) have noted that high variability in a parameter such as growth or fecundity among individual fish can result in low sensitivity of a particular test endpoint when within-replicate variation is employed as the error term in tests of significance. When using a hypothesis-testing approach as described by Mount and Stephan (1967), high parameter variability will mean

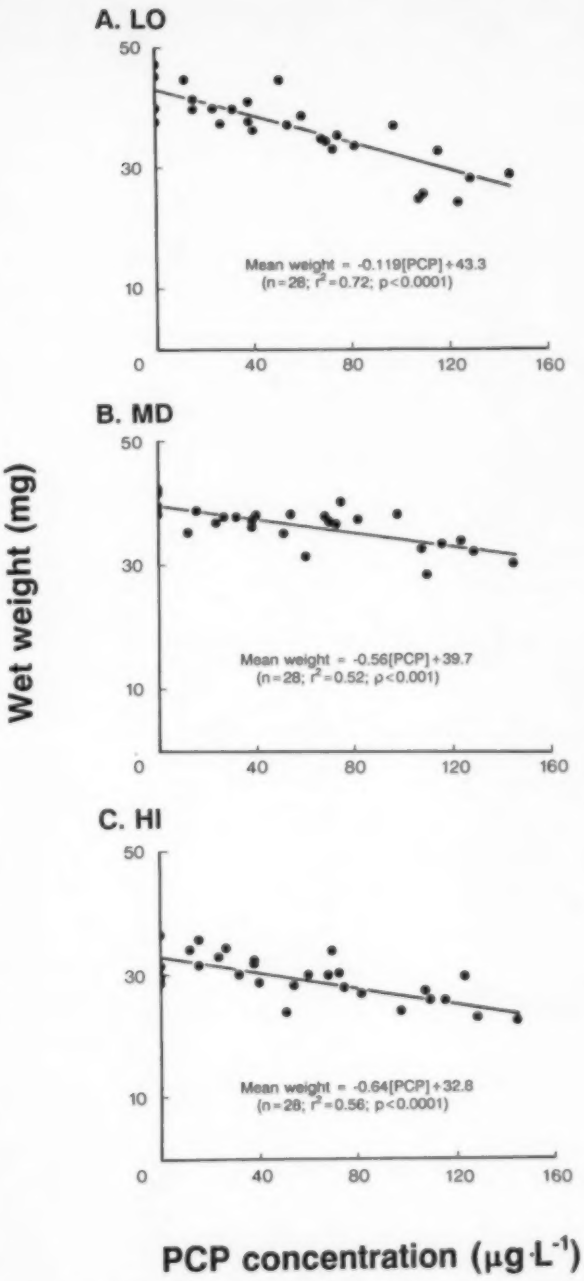

FIG. 1. Effect of a 28-d exposure to PCP on the growth of fathead minnow at (A) LO, (B) MD, or (C) HI rearing densities. The mean weight of the fish in each replicate (tank) is plotted against the mean assayed PCP concentration for that replicate.

that a greater real effect is necessary to detect a significant difference. If the data are subjected to regression analysis, and a percent-inhibition concentration calculated, high variability will result in large confidence limits on the toxicity estimate. Based on the results of our study, individual variation in growth of fish at different rearing densities would have an obvious effect on endpoint calculation if within-replicate variation in fish weight was used to test for effects on fish growth. The most appropriate tests of significance use the mean replicate weight and the among-replicate variation, accepted practice in standardized fathead minnow growth and survival tests (USEPA 1989; Environment Canada 1992).

The density-related effects on growth reported here cannot be explained by statistical anomalies due to differences in individual variation at the different density levels; some interaction between toxicant stress and rearing density resulted in a real biological change in the response to the toxicant. In other words, the density at which the fish were held altered the nature of the concentration–response relationship. The effect of PCP exposure was reduced with increasing fish density. The change was more dramatic for TCP in that the actual shape of the concentration–response curve changed with density. Although we have no ready explanation, the quadratic nature of the relationship for the LO- and MD-density fish may have been due to hormesis (growth stimulation) at low concentrations. In a review of chronic toxicity test data, Suter et al. (1987) noted that apparent hormesis complicated the analysis of many of the results examined. Alternatively, the nonlinear nature of the response curve may reflect a true threshold concentration for effects on growth; analysis of residuals from the regression analysis indicated an abrupt change in the response curve, similar to a breakpoint between linear trends.

It is possible that the density-related effects reported here were due to a reduction (presumably through metabolism) in the toxicant concentration in the immediate vicinity of the fish, a reduction that would have been greater in the HI- versus the LO-density treatments. This would lead to less apparent toxicity in the HI-density fish. This phenomenon has been observed in static test systems using small test volumes (Sprague 1969). We do not support such an explanation for these results. Although toxicant concentrations were not measured to allow direct comparisons of actual concentrations in different rearing cages, the system was designed to provide suitable test volumes, adequate replenishment, and good circulation between cages. Based on final fish weights, the maximum loading of fish in each test chamber at HI density was ≈0.5 g fish·L^{-1}. The 95% molecular replacement time for the system was <7 h (Sprague 1969); test solution was provided at a rate of >100 L·g fish^{-1}·d^{-1}. Dye studies on the exposure system completed prior to testing indicated good mixing and even distribution of toxicant throughout each tank and its cages. Given the respective bioconcentration factors of approximately 400 and 150 for PCP and TCP measured during these studies (Arthur 1991), and the high rate of replenishment, any local reductions in concentrations that may have occurred are considered to be negligible. We are confident that the observed effects are related to density-dependent changes in the concentration–response relationship, and not to local changes in concentration caused by different fish densities.

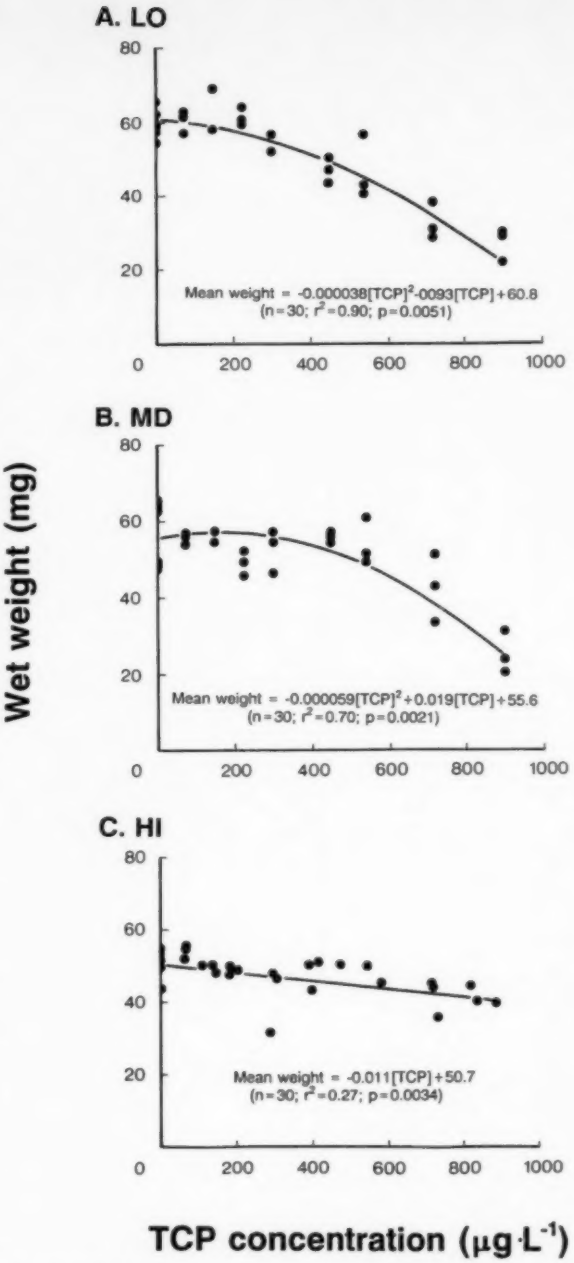

FIG. 2. Effect of a 28-d exposure to TCP on the growth of fathead minnow at (A) LO, (B) MD, or (C) HI rearing densities. The mean weight of the fish in each replicate (tank) is plotted against the mean assayed PCP concentration for that replicate.

Irrespective of the reason for the differences, different rearing densities resulted in as much as twofold differences in chronic toxicity estimates based on our growth studies. It is apparent that rearing density should be carefully considered in the development of standard protocols for chronic toxicity tests with larval and juvenile fish.

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Acute and Chronic Toxicity of Waterborne Arsenite to Rainbow Trout (*Oncorhynchus mykiss*)

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Rankin, M.G., and D.G. Dixon. 1994. Acute and chronic toxicity of waterborne arsenite to rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 51: 372–380.

The 144-h LC50 (95% fiducial limits) of arsenite for 4.5-g rainbow trout (*Oncorhynchus mykiss*) was 18.5 (17.9–19.1) mg·L^{-1}. In a pair-fed growth study which exposed trout to 0.0, 0.76, 2.48, or 9.64 mg arsenite·L^{-1} over 17 wk, growth was significantly reduced (by 55%) only at 9.64 mg·L^{-1}. The reduction was attributable to both reduced appetite (primarily) and direct metabolic impact (marginally). Fish at 9.64 mg·L^{-1} suffered 10% mortality, usually associated with necrotic erosion of the mandibular and olfactory regions of the head. All fish exposed to 9.64 mg·L^{-1} showed inflammation of the gallbladder wall, a lesion absent at lower exposure concentrations. There were no arsenite impacts on hepato- and splenosomatic index, hematocrit, hemoglobin, total plasma lipid, cholesterol, and protein or brain concentrations of norepinephrine, dopamine, and serotonin. Exposure to 0.0, 0.76, 2.48, and 9.64 mg arsenite·L^{-1} for 26 wk resulted in mean (SE) equilibrium whole-body As concentrations of 0.3 (0.02), 0.2 (0.02), 0.4 (0.10), and 1.7 (0.40) μg As·g wet weight^{-1}, respectively, No depuration below these concentrations occurred during a 12-d period in arsenite-free water. The threshold of chronic toxicity was estimated to be 4.9 mg·L^{-1}.

La CL_{50}-144 h (limite de confiance de 95 %) de l'arsénite pour une truite arc-en-ciel (*Oncorhynchus mykiss*) de 4,5 g était de 18,5 (17,9 à 19,1) mg·L^{-1}. Dans le cadre d'une étude de croissance portant sur des truites nourries ensemble où celles-ci étaient exposées à des concentrations de 0,0, 0,76, 2,48 ou 9,64 mg arsénite·L^{-1} pendant 17 sem, la croissance était significativement réduite (de 55 %) seulement lorsque la concentration était de 9,64 mg·L^{-1}. La réduction était attribuable à une diminution de l'appétit (principalement) et à un effet métabolique direct (secondairement). Le taux de mortalité des poissons exposés à 9,64 mg·L^{-1} était de 10 %, en général associé à une érosion nécrotique des régions mandibulaire et olfactive de la tête. Tous les poissons exposés à une concentration de 9,64 mg·L^{-1} présentaient une inflammation de la paroi de la vésicule biliaire, lésion absente lorsque les organismes sont exposés à des concentrations plus faibles. On a relevé aucun effet de l'arsénite sur l'indice hépato-splénosomatique, l'hématocrite, l'hémoglobine, les lipides plasmatiques totaux, le cholestérol et les protéines, ou les concentrations de norépinéphrine, de dopamine et de sérotonine dans le cerveau. Une exposition à 0,0, 0,76, 2,48 et 9,64 mg arsénite·L^{-1} pendant 26 sem s'est traduite par des concentrations moyennes (erreur-type) à l'équilibre d'arsénite dans tout le corps, respectivement de 0,3, (0,02), 0,2 (0,02), 0,4 (0,10) et de 1,7 (0,40) μg As·g (poids frais)$^{-1}$. Il ne s'est produit aucune dépuration au-dessous de ces concentrations pendant une période de 12 jours dans une eau exempte d'arsénite. Le seuil de toxicité chronique a été évalué à 4,9 mg·L^{-1}.

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Arsenic (As), a metalloid, is ubiquitous in the environment. Natural sources of As to aquatic ecosystems include weathering and erosion of rock, while anthropogenic sources include mining, roasting and smelting of ores, and coal-fired electric power generation (NRCC 1978). Although total As concentrations in surface waters are generally less than 10 μg·L^{-1}, and typically on the order of 1 μg·L^{-1}, concentrations can range from 250 to 5500 μg·L^{-1} in waters impacted by mining activity (Wagemann et al. 1978; Mudroch and Capobianco 1980). The current Canadian water quality guideline for As is 50 μg·L^{-1} (CCREM 1987).

Based on redox potential (E_h), four valence states of inorganic As (3−, 0, 3+, and 5+) can theoretically exist in freshwater systems, although As in the 3− or 0 state is rare. In oxygenated waters under normal E_h conditions, As^{5+} (arsenate) is prevalent, while decreases in pH and E_h favour a greater proportion of As^{3+} (arsenite) (Ferguson and Gavis 1972). Because arsenate predominates in oxygenated waters, the majority of recent toxicological research on As with aquatic organisms has centered on arsenate, even though arsenite is generally considered to be more toxic, at least at the acute level during waterborne exposure (McGeachy and Dixon 1989, 1992). Recently, however, several authors (Seyler and Martin 1989, 1991; Anderson and Bruland 1991) have reported considerably higher concentrations or arsenite in oxygenated waters than would be expected based on thermodynamic equilibria. In Moira Lake, an As-contaminated system in Ontario, As in the hypolimnion is largely present as arsenite (>70%); despite the thermodynamic predictions, ≈25% of the As in the epilimnion is also present as arsenite (Azcue 1992). Azcue suggested that the high concentrations of arsenite could be attributed either to the upward diffusion of arsenite from the interstitial water of sediments or to biologically mediated arsenite formation. Based on the pre-

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viously underestimated potential for arsenite bioavailability in the natural environment, work in our laboratory has recently focused on the toxicity of both arsenate and arsenite to rainbow trout (*Oncorhynchus mykiss*) during long-term chronic exposure. We have previously reported on the chronic toxicity or arsenate (McGeachy and Dixon 1990); this paper presents the results of parallel work on arsenite.

It is clear that As can inhibit growth performance in aquatic organisms (Gilderhus 1966; Call et al. 1983; Lima et al. 1984). In those studies which used arsenite, however, exposures were typically less than 1 mo in duration, and no effort was made to differentiate between appetite depression and direct impacts on metabolism. This study was initiated, in part, to explore longer term impacts, and particularly to partition appetite depression and bioenergetic effects.

One of the most sensitive indicators of As toxicity seems to be altered behaviour., Weir and Hine (1970) reported that a 24-h exposure to 100 μg arsenate·L^{-1} impaired a conditioned avoidance response in goldfish (*Carassius auratus*). Some workers have explored the possibility of using brain amine concentrations as sensitive indicators of altered neurochemistry and hence (potentially) of behaviour (Holdway et al 1986; Sloley et al. 1986). To this end, we monitored changes in the concentrations of three brain amines during arsenite exposure.

The final area of emphasis in this work involves relating toxicological responses by exposed fish to their whole-body As concentration. Considerable attention has recently been placed on the use of the critical whole-body concentration (CWBC) concept in aquatic toxicology. The concept is based on the assumption that the internal toxicant concentration which illicits a biological response will be more closely related to the response, and subject to less variability induced by modifying factors, than the corresponding aqueous exposure concentration. Connolly (1985) suggested that relationships between the CWBC of a chemical and specific endpoints of toxicity should be independent of the majority of toxicity modifying factors and, therefore, more transferable from the laboratory to the aquatic environment. A number of authors have attempted to increase the predictive ability of water quality guidelines by determining internal threshold concentrations of toxicants which elicit specific responses for specific chemicals (Van Hoogen and Opperhuizen 1988; McCarty et al. 1989, 1992). In view of this, we followed the whole-body As concentration in fish during exposure and subsequent depuration.

Materials and Methods

This research was initiated with a 144-h acute toxicity test which exposed rainbow trout to six concentrations of arsenite (nominally 0, 10.7, 14.4, 18.0, 21.2, and 30.0 mg·L^{-1}) in duplicate. The acute toxicity test was followed by a separate 181-d chronic study which exposed trout to nominal arsenite concentrations of 0.5, 2.0, or 8.0 mg·L^{-1}. During the chronic study, each exposure was performed in triplicate using a pair-fed design where each treated tank was paired with an unexposed control tank for a total of 18 tanks (3 concentrations × 2 controls × 3 replicates). Food was provided to the total biomass of each paired control tank to match the daily food consumption of the fish in the corresponding paired exposure tank, thus making it possible to distinguish arsenite growth effects due to appetite suppression from direct physiological impacts. The effects of chronic exposure were assessed in terms of mortality, wet weight, condition factor, hepato- and splenosomatic indices, hematocrit, hemoglobin, total plasma lipid cholesterol, and protein, concentrations of three brain amines (norepinephrine, serotonin, and dopamine), whole-body As concentration (including concentrations during post-exposure depuration), and the presence or absence of histopathological lesions in gill, heart, kidney, liver, gall bladder, spleen, muscle, and skin tissue.

For the acute toxicity test, rainbow trout weighing from 4 to 6 g were obtained from Shamrock Springs Trout Hatchery, Thamesford, Ont. They were held in 200-L continuous-flow (1 L·min^{-1}) tanks at 10°C for 1 wk prior to the initiation of the toxicity tests. During this time, they were fed a commercial juvenile salmonid diet (40% protein, 3% cellulose, 12% crude fat) at a rate of 3% wet weight·d^{-1}. The acclimation photoperiod, used throughout the study, was 12 h light : 12 h dark with 0.5 h of artificial dusk and dawn included in the light portion of the cycle. Fish for the chronic study (initial wet weight, 2.5–3.5 g) were obtained from Spring Valley Trout Farm, Petersburg, Ont., and held as outlined above at 13°C.

The exposure apparatus for both the acute and chronic studies consisted of eighteen 76-L aerated rectangular glass aquaria adjusted to 60 L with standpipes. Arsenite exposure solutions of the required concentration were delivered to the tanks by a modified Mount and Brungs diluter (Lemke et al. 1978). The flow rate to each tank was maintained at 180 mL·min^{-1} for a 90% molecular replacement time of 0.5 d.

Arsenite stock solutions were prepared with arsenic trioxide (As_2O_3 >98% pure, Sigma Chemical Co., St. Louis, Mo.). The appropriate amount of As_2O_3 was dissolved in 30 L of 0.5% NaOH (w:v) and deionized water was added to bring the volume to 120 L. Concentrated HCl was added to neutralize the NaOH and bring the pH to 6.5, thus reducing the potential for oxidation of arsenite to arsenate. The stock solution was adjusted to a final volume of 125 L, capped to further minimize the potential for oxidation, and held at room temperature. Under these conditions, As in solution will exist primarily as the triprotonated species of arsenous acid, although the diprotonated species may also be present (Ferguson and Gavis 1972). A full characterization of the groundwater used for dilution throughout this project is given in Kevan and Dixon (1991). Samples were taken from randomly selected exposure tanks daily during the acute toxicity test, and every third day during the chronic study, for analysis of dissolved oxygen (Winkler titration; APHA 1980), total hardness (EDTA titration; APHA 1980), alkalinity (HCl titration; APHA 1980), temperature, and pH. The mean (SD) water conditions during the acute toxicity test were as follows: dissolved oxygen, 9.6 (0.2) mg·L^{-1}; total hardness, 373 (3) mg·L^{-1} as $CaCO_3$; alkalinity, 273 (2) mg·L^{-1} as $CaCO_3$; temperature, 13.8 (0.2)°C; and pH, 8.0 (0.02). The mean (SD) conditions during the chronic exposure were as follows: dissolved oxygen, 7.8 (0.2) mg·L^{-1}; total hardness, 380 (3) mg·L^{-1} as $CaCO_3$; alkalinity, 282 (3) mg·L^{-1} as $CaCO_3$; temperature, 13.4 (0.2)°C; and pH, 7.8 (0.06). Subsurface water samples were collected daily from each tank during the acute toxicity test, acidified to 1% with analytical grade nitric acid, and stored for determination of total As concentration by neutron activation analysis

TABLE 1. Formulation of the semipurified experimental diet used during the 181-d chronic exposure of rainbow trout to arsenite.

Ingredient	Proportion (% by weight)
Casein	45
Gelatin	4
Starch	20
Alpha-floc	5
Fish oil	15
Amino acid premix[a]	1
Vitamin premix[b]	2
Mineral premix[c]	8

[a]Providing L-arginine HCL (0.2%), DL-methionine (0.3%), and starch filler (0.5%).

[b]Providing (to meet or exceed requirements) vitamins A, B_{12}, D, E, and K, thiamine, riboflavin, niacin, pridoxine, pantothenic acid, folic acid, biotin, inositol, choline, ascorbic acid, and butylated hydroxytoluene.

[c]Providing (to meet or exceed requirements) $CaHPO_4 \cdot H_2O$, $CaCO_3$, NaCl, K_2SO_4, $MgSO_4$, ferric citrate, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot H_2O$, $CuSO_4 \cdot 5H_2O$, $CoCl_2 \cdot 6H_2O$, and KI.

(McGeachy and Dixon 1989). Mean in-tank As concentrations ranged from 99 to 108% of nominal with a mean coefficient of variation (CV) of 10.3. Samples were taken every third day during the chronic exposure; As concentration ranged from 96 to 152% of nominal with a mean CV of 14.2. During both studies, control tanks were sampled on a rotating basis; the background concentration never exceeded the detection limit of 0.02 mg $As \cdot L^{-1}$. All results in this paper are reported in terms of assayed As concentration.

Prior to the acute toxicity test, 120 trout were randomly allocated to 12 of the exposure tanks (10 per tank) where they underwent a further 21-d acclimation period at 13°C on the commercial diet described above. To start the test, the tanks were inoculated with sufficient stock solution to bring the arsenite levels to the desired nominal concentrations. The diluter was then engaged to maintain the toxicant concentrations during the test. This technique for initiating exposure was preferred (relative to dropping the fish into preestablished concentrations) because it eliminated netting and handling stress prior to exposure. The fish were not fed during the test, which lasted for 144 h with mortalities recorded at 0, 0.5, 1, 2, 4, 8, 16, 24, 36, 48, 60, and 76 h, and every 24 h thereafter.

Three weeks prior to the chronic study, 60 fish were randomly allocated to each of the 18 tanks. For the first week, they were fed the commercial salmonid diet. At the start of the second week the original diet was replaced with a semipurified experimental diet formulated at the Department of Nutritional Sciences, University of Guelph (Table 1). The diet was mixed in 20-kg batches, steam-pelleted, and crumbled to size as required. After 2 wk on the new diet, arsenite exposure was initiated as outlined for the acute toxicity test. Throughout the exposure, the total wet weight of the fish in each tank was determined weekly to monitor changes in wet weight and to determine (and modify) the feeding rate. The fish were counted into a polyethylene colander held in water. The colander was weighed with and without fish to give (by difference) the total biomass of fish per tank. The fish were then returned to the exposure tanks. The fish were fed three times a day. As a guide, rations of 3% total biomass were prepared daily. Food was provided to match the vigour of the feeding response (excess food was avoided); the last daily feeding was adjusted such that consumption by controls matched that of their paired exposure tank. Mortalities (recorded daily) over the 121-d growth portion of the chronic study were expressed as a percentage of the initial number of fish in each tank (60). The percentages were not corrected for the sequential removal of fish for determination of whole-body As concentration as outlined below.

After 17 wk of exposure (the end of the growth portion of the chronic study), five to seven fish were sampled from each tank (15–21 fish per treatment) for the determination of condition factor (K), hepatosomatic index (HSI), splenosomatic index (SSI), and a number of haematological parameters. The fish were anaesthetized with tricaine methanesulphonate and blood was collected by caudal puncture into either heparinized microhematocrit tubes (for determination of hematocrit by centrifugation) or 5-mL heparinized Vacutainers (Becton-Dickenson, Rutherford, N.J.). A portion of the whole blood was used for determination of total hemoglobin (Unopette cyanmethemoglobin kit; Becton-Dickenson, Rutherford, N.J.). The remainder was centrifuged and the plasma stored at −196°C pending analysis for total lipids (sulfophosphoranillin reaction kit; Boehringer-Mannheim Co. Ltd., Montreal, Que.), total cholesterol (peroxidase kit; Fisher Scientific Co. Ltd., Ottawa, Ont.), and total protein (Biuret kit; Sigma Chemical Co., St. Louis, Mo.). The length and total weight, as well as the liver and spleen weights, were recorded for each fish. The K was calculated as $100(\text{weight} \cdot \text{length}^{-3})$ and HSI and SSI as $100(\text{organ weight} \cdot \text{body weight}^{-1})$.

Fish were also sampled at 17 wk for determination of the concentrations of three neuroamines (norepinephrine, dopamine, and serotonin) in brain tissue. Two fish per tank (six per treatment) were killed by cervical dislocation. The head of each fish was bisected sagitally; the brain was removed and stored at −196°C pending analysis. Brain amine concentrations were assayed by HPLC with electrochemical detection using methods reported by Holdway et al. (1986). An additional three fish were taken from each tank (15 per treatment) and portions of the gill, heart, liver, gall bladder, kidney, spleen, and dorsal muscle (with attached skin) were fixed in buffered formalin for histological examination. The preserved tissues were embedded in paraffin, section (9 μm), and stained with haematoxylin and eosin using standard methods (Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ont.). The slides were coded and randomized prior to qualitative examination by light microscope.

The whole-body As concentration of fish was monitored throughout the 181-d arsenite exposure. On days 0, 1, 2, 4, 8, 17, 55, 127, and 181, three fish per tank (nine per treatment) were removed, killed by cervical dislocation, weighed, rinsed in deionized water, individually bagged, and stored frozen (−20°C) pending determination of whole-body As concentration by neutron activation analysis as outlined by McGeachy and Dixon (1989). After the final sampling at 181 d of exposure, depuration was initiated by pooling 35 fish from each treatment and placing them in aquaria receiving arsenite-free water. Five fish per treatment were sampled (as above) on days 0, 0.5, 1, 2, 4, 6, and 12.

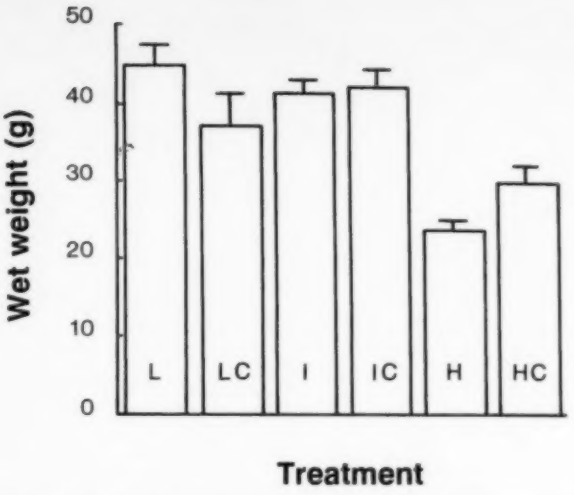

FIG. 1. Wet weight of rainbow trout at the end of a 121-d exposure to a low (L, 0.76 mg As·L^{-1}), intermediate (I, 2.48 mg As·L^{-1}), or high (H, 9.64 mg As·L^{-1}) concentration of arsenite. LC, IC, and HC are the weights of the respective pair-fed controls. Values are given as the grand mean of the three replicates, with bars indicating the SE.

Mortality data for the acute toxicity test were analyzed using probit analysis (Finney 1971). Correction for control mortality was not necessary; none occurred. The resulting LC50s were compared using the standard error of the difference (Sprague and Fogels 1977). Results for the wet weight of fish at 121 d of arsenite exposure, *K*, HSI, SSI, neuroamine concentrations, and the various blood and plasma parameters were characterized using two-way analysis of variance (ANOVA). Fish weights over the course of the growth study were assessed by regressing weekly weight (tank biomass) against time for each treatment and comparing the slopes and intercepts by analysis of covariance (ANCOVA). Whole-body As concentrations were analyzed by one-way ANOVA. After completion of the ANOVAs, means were compared (where applicable) using *T*, *T'*, or Tukey-Kramer tests (Steele and Torrie 1980). All tests were performed with PC-based SYSTAT (version 5.0; Systat Inc., Evanston, Ill.) data analysis software. When necessary, data were transformed prior to analysis to achieve homoscedasticity.

Results

The 96- and 144-h LC50s (95% fiducial limits) of arsenite to rainbow trout were, respectively, 21.0 (19.7–22.3) and 18.9 (17.9–19.9) mg As·L^{-1} for replicate 1 and 20.2 (18.8–21.4) and 18.1 (17.4–18.8) mg As·L^{-1} for replicate 2. The replicate LC50s were not different ($p > 0.05$) at 96 or 144 h. The pooled 144-h LC50 of 18.5 (17.9–19.1) mg As·L^{-1} was, however, lower ($p > 0.05$) than the pooled 96-h LC50 of 20.6 (20.0–21.5) mg As·L^{-1}; mortality was still changing between 96 and 144 h. As such, a threshold was not attained and the 144-h LC50 does not represent an incipient lethal level. Complete mortality data for all exposure times from 16 to 144 h are available in Rankin (1987).

The pooled-treatment-mean (SD, *n*) exposure concentrations during the chronic toxicity study were 0.76 (0.17, 73), 2.48 (0.69, 68), and 9.64 (1.82, 72) mg As·L^{-1}. There were no significant differences in mean As concentration among replicates within treatments. Mortality during the chronic study averaged less than 1% per tank, with the exception of the three replicates at 9.64 mg As·L^{-1} where from 7 to 13% of the fish died. The mortality consisted of fish with no overt signs of toxicity (2–3%) and fish which exhibited severe erosion of the mandibular and olfactory region (4–10%). The lesion, which first appeared at around 30 d of exposure, was characterized by a discolouration and loss of tissue around the distal portion of the lower mandible and/or the nares. Tissue loss progressed until the mandible and the region surrounding the nares were eroded to the point where skeletal material was visible. Once the lesion had reached this stage, fish typically survived for 1–2 wk. Overall, the progress of the lesion spanned approximately 4 wk from initial onset to mortality. None of the fish from either the control or the lower arsenite exposure treatments exhibited these signs.

TABLE 2. Comparison of the slopes and intercepts of regressions of wet weight (weekly tank biomass) against time for rainbow trout exposed to one of three concentrations of arsenite (L, I, H) for 121 d. Within columns, values followed by a common italicized letter are not significantly different ($p > 0.05$). All of the regressions were significant ($p < 0.001$) and had $r^2 \geq 0.98$.

Treatment[a]	Log slope	Log intercept
LC	0.0089 *a*	0.589 *a*
L	0.0093 *a*	0.587 *a*
IC	0.0091 *a*	0.565 *b*
I	0.0091 *a*	0.574 *b*
HC	0.0080 *b*	0.522[b]
H	0.0074 *c*	0.496[b]

[a]See Fig. 1 for explanation of codes.
[b]Comparison of these intercepts is inappropriate, since the slopes are significantly different.

Prior to the start of exposure, all fish were feeding with equal vigour. At the highest exposure concentration the feeding response was noticeably affected within 1 d; no feeding frenzy was apparent and the fish generally lacked enthusiasm and vigour when food was offered. Because this behaviour was consistent with all replicates at the high exposure concentration, the food ration was reduced; the ration provided to the pair-fed controls was reduced accordingly. Treated and control fish at the two lower exposure concentrations did not differ in feeding response throughout the study.

The wet weights of the rainbow trout at the end of the 121-d study (Fig. 1) were analyzed using two-way ANOVA with control treatment (exposed versus pair-fed controls) and exposure concentration as the two factors. The final wet weight of the pair-fed controls at 9.64 mg As·L^{-1} was reduced ($p < 0.05$) relative to the other control groups due to the imposed reduction in food ration. Although the control treatment effect (exposed versus pair-fed controls) was not significant ($p = 0.68$), there was a large exposure concentration effect ($p < 0.001$) and the interaction between exposure concentration and control treatment was marginally significant ($p = 0.05$). Orthogonal comparisons of individual

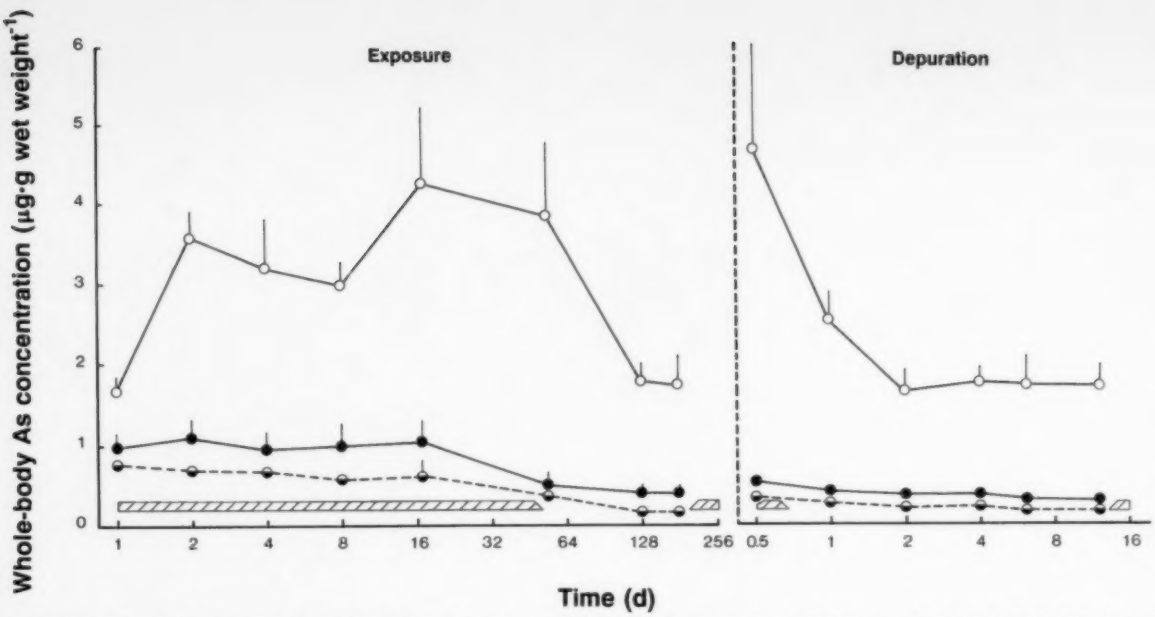

FIG. 2. Whole-body As concentrations shown by rainbow trout during a 181-d exposure to 9.64 (open circles), 2.48 (solid circles), or 0.76 (half-solid circles) mg arsenite·L^{-1}, followed by 12 d of depuration in arsenite-free water. Each point is a mean with the SE. Where no error bar is given, the SE falls within the diameter of the point. The hatched band is the mean ± SE for 18 control fish sampled periodically during the exposure.

TABLE 3. Mean (SD) condition factor (K), hepatosomatic index (HSI), and splenosomatic index (SSI) for rainbow trout after a 121-d exposure to one of three concentrations of arsenite. For K, values followed by a common italicized letter are not significantly different ($p > 0.05$). There were no significant treatment effects on either HSI or SSI.

Treatment[a]	n	K	HSI	SSI
LC	15	0.98 (0.09) *a*	0.82 (0.19)	0.21 (0.11)
L	15	1.02 (0.08) *a*	0.84 (0.20)	0.24 (0.09)
IC	15	1.02 (0.04) *a*	0.82 (0.18)	0.26 (0.09)
I	15	1.01 (0.08) *a*	0.85 (0.22)	0.25 (0.13)
HC	10	0.98 (0.05) *a*	0.84 (0.11)	0.24 (0.06)
H	13	1.13 (0.08) *b*	1.03 (0.36)	0.22 (0.08)

[a]See Fig. 1 for explanation of codes.

exposure concentrations versus their respective controls revealed no arsenite effect on final wet weight at the low ($p = 0.10$) or intermediate ($p = 0.77$) concentrations but a reduction ($p = 0.05$) at the highest exposure concentration.

Fish weights (weekly tank biomass) taken over the course of the study were pooled according to treatment, log transformed, regressed with respect to time, and the slopes and intercepts tested for differences among treatments using ANCOVA (Table 2). The regressions for the two lower exposure concentrations and their respective controls were similar in slope ($0.25 < p < 0.50$) although the log intercepts differed ($0.001 < p < 0.005$) (i.e., separate but parallel lines). The regressions for the highest exposure concentration and its control were different from the rest of the relationships and from each other ($0.01 < p < 0.05$).

Arsenite exposure for 121 d had limited effect on K and no significant impact on HSI or SSI (Table 3). For K, there was a significant exposure concentration effect ($p = 0.03$) and an interaction between exposure concentration and control treatment ($p = 0.001$). Subsequent post hoc tests indicated that the K for the highest arsenite exposure concentration was higher ($p < 0.01$) than the K of all other groups. For HSI, there was no significant exposure concentration effect ($p = 0.20$), control treatment effect ($p = 0.13$), or interaction between the two ($p = 0.44$). The SSI results showed the same outcome for exposure concentration ($p = 0.44$), control treatment ($p = 0.94$), and interaction ($p = 0.60$).

During the growth period, there were no significant impacts of arsenite exposure on a number of blood parameters (Table 4) including hematocrit ($p = 0.76$), hemoglobin ($p = 0.16$), total plasma lipids ($p = 0.11$), plasma cholesterol ($p = 0.26$), and plasma protein ($p = 0.66$). There was a similar absence of arsenite exposure effect on the relative concentrations of three neuroamines (norepinephrine, dopamine, and serotonin) in brain tissue (Table 5). The concentrations of each amine were assessed using two-way ANOVA with control treatment and exposure concentration as the factors. There was no effect of either factor on any of the amine concentrations ($p > 0.51$ for all comparisons). In addition, none of the interaction terms were significant ($p > 0.14$).

Necropsies of fish exposed to 9.64 mg arsenite·L^{-1} revealed that their gallbladders were smaller in size and had opaque walls, compared with gallbladders from either the controls or the other arsenite treatment groups. Histological examination of these gallbladders showed that (relative to controls) the lamina epithelialis was highly involuted and the lamina muscularis was extensively thickened. The lamina muscularis was also edematous and nectotic, particularly toward the outer (away from the lumen) periphery. In addition to the above, the gallbladder lumen was filled with inspissated (thickened or jellylike) bile. Although the gallbladder lesions were apparent to some extent in all of the fish exposed to 9.64 mg arsenite·L^{-1}, they did not appear in any

TABLE 4. Mean (SD, n) hematocrit (HT), hemoglobin (Hb), total plasma lipids (TPL), plasma cholesterol (PC), and plasma protein (PP) shown by rainbow trout after 121-d of exposure to one of three concentrations of arsenite. There were no significant ($p > 0.05$) treatment effects on any parameter.

Treatment[a]	HT (%)	Hb ($g \cdot dL^{-1}$)	TPL ($mg \cdot dL^{-1}$)	PC ($mg \cdot dL^{-1}$)	PP ($g \cdot dL^{-1}$)
LC	34.6 (4.9, 15)	5.36 (0.62, 15)	809 (96, 10)	178 (12, 20)	2.65 (0.19, 10)
L	35.6 (6.2, 12)	5.42 (0.97, 14)	917 (70, 10)	201 (10, 20)	2.77 (0.12, 10)
IC	31.4 (4.3, 11)	5.24 (0.99, 15)	651 (65, 10)	161 (7, 20)	2.80 (0.24, 10)
I	29.6 (6.5, 9)	4.97 (0.94, 9)	636 (81, 9)	150 (11, 20)	2.36 (0.22, 10)
HC	34.0 (2.6, 5)	5.83 (0.90, 12)	749 (58, 7)	182 (11, 14)	2.76 (0.12, 10)
H	33.4 (4.2, 5)	5.08 (1.38, 14)	706 (88, 9)	171 (13, 22)	2.63 (0.19, 10)

[a]See Fig. 1 for explanation of codes.

other control or exposed group. Histological examination revealed no lesions in the gills, heart, kidney, liver, spleen, muscle, or skin tissue of any treatment group.

During the 181-d kinetics study, whole-body As concentration could be correlated with arsenite exposure level as early as day 1 (Fig. 2). For all three treatments, As content reached a preliminary peak within 2 d of exposure, followed by a temporary decline. A subsequent rise in As content was detected at 17 d of exposure. Following this secondary peak, a gradual decline was evident until 127 d; concentrations did not change ($p > 0.05$) between 127 and 181 d for any of the three exposure levels. The whole-body As concentration in fish exposed to 9.64 mg arsenite$\cdot L^{-1}$ was consistently ($p < 0.05$) higher than the concentration in fish from the two lower exposure levels. Fish exposed to 2.48 mg arsenite$\cdot L^{-1}$ had body concentrations marginally but significantly ($p < 0.05$) greater than those evident at the lowest exposure level beyond day 2. Concentrations in fish held at 0.76 mg arsenite$\cdot L^{-1}$ were significantly elevated relative to the 0.27 μg As$\cdot g^{-1}$ present in control fish until day 16, after which the concentrations were not significantly elevated over background.

The depuration of As, initiated after 181 d by transferring the fish to arsenite-free water, was monitored for 12 d (Fig. 2). The As content in fish at 181 d was taken as the baseline for 0 d of depuration. An unexpected pattern emerged that was consistent and temporally synchronized across all three exposure levels. Twelve hours after transfer, fish from the high, intermediate, and low exposure groups showed respective increases in mean As concentration of 176, 9, and 67% over the appropriate 181-d (0 d of depuration) concentrations. The increases were significant ($p < 0.05$) at the high and low exposure levels. This increase was still present, although to a lesser degree, after 1 d. By 2 d, the whole-body As concentrations returned to values consistent with concentrations at 0 h of depuration; further decreases did not occur over the subsequent 10 d.

Discussion

The 144-h LC50 of 18.5 mg arsenite$\cdot L^{-1}$ for 5-g rainbow trout at 14°C given here is consistent with the values of 17.7 and 20.2 mg$\cdot L^{-1}$, at 5 and 15°C respectively, for trout of similar size previously reported by our laboratory (McGeachy and Dixon 1989) and with other literature values for salmonids (Buhl and Hamilton 1991).

Significant levels of mortality did not occur during the chronic study until approximately 4 wk after the start of exposure, and then only at the highest exposure concentration. Mortality associated with the rostral lesion was not evident until 6 wk of exposure; the incidence of mortality where the lesion was not evident decreased as the study progressed. The early stages of the lesion reported here were similar to those reported by Cardwell et al. (1976) during exposure of rainbow trout to acutely lethal concentrations of arsenite. The complete etiology of the condition is currently unclear, although we suspect that it may be associated with loss of blood circulation to the rostral region. A parallel syndrome can occur in humans chronically exposed to As (Tseng 1977): during the progress of the condition, initially the toes, and in severe cases the entire foot, can be lost due to necrosis resulting from loss of blood flow induced by an As-mediated arteriosclerosis.

A significant reduction in final wet weight occurred only at the 9.64 mg arsenite$\cdot L^{-1}$ exposure concentration. This result is supported by the regression and ANCOVA analysis of the weight data through time: only fish at the highest exposure concentration were impacted. Although the dramatic reduction in growth at this concentration could partially be attributed to a direct metabolic impact of arsenite, the major portion of the reduction was due to reduced food consumption. Arsenite depressed food intake at 9.64 mg$\cdot L^{-1}$ as early as day 1 of exposure. Throughout the study, food consumption approximated 1% wet weight relative to the 3% offered. Because the response was essentially immediate, the behaviour may have been mediated through chemoreception of the toxicant. Conversely, in view of the rostral lesion, the depression of feeding activity may have been associated

TABLE 5. Mean (SD) concentrations (ng$\cdot$g wet brain weight^{-1}) of norepinephrine (NE), dopamine (D), and serotonin (S) in brain tissue of rainbow trout after 121-d of exposure to one of three concentrations of waterborne arsenite. All of the means are based on a sample size of six. There were no significant ($p > 0.05$) treatment effects on any parameter.

Treatment[a]	NE	D	S
LC	591 (106)	302 (39)	291 (45)
L	589 (60)	263 (84)	296 (59)
IC	569 (99)	303 (20)	332 (52)
I	584 (64)	293 (48)	303 (27)
HC	641 (41)	314 (57)	311 (58)
H	647 (165)	348 (124)	373 (77)

[a]See Fig. 1 for explanation of codes.

with a loss of chemoreception, and thus the fish's ability to smell and taste food. Copper-induced appetite depression in rainbow trout (Waiwood and Beamish 1978) has been linked to histological damage to the oral cavity (Eisler and Gardner 1973) and taste receptors (Hidaka 1970).

The *K*, an indicator of the general nutritional state of fish (Weatherby 1972), was significantly elevated at the highest exposure concentration relative to all other groups. This was an unexpected result; it infers a better nutritional state and conflicts with the fact that these fish consumed a smaller ration due to appetite suppression. One plausible explanation is that the significant number of mortalities at this level of exposure may have selected against the weaker fish. This differential selection could have skewed the distribution of *K* among the remaining fish, resulting in a biased sample.

Somewhat surprisingly, none of the other indicators of bioenergetics and metabolism which we assayed showed arsenite impact. Although elevated plasma protein has been associated with chronic As toxicity in mammals (WHO 1981), there was no significant impact here; all values fell within the range considered normal for salmonids (Miller et al. 1983). A reduction in either cholesterol or total plasma lipids can signify impaired lipid metabolism (Wedemeyer and Yasutake 1977). In the present study, both parameters were consistent with levels considered normal for rainbow trout (Hille 1982). The absence of impact on plasma cholesterol at the highest exposure concentration is interesting in light of the gallbladder pathology evident in these fish. These gallbladders suggest reduced bile production based on their atrophied state and the presence of inspissated bile. Hepatic cholesterol is incorporated into bile salts and is passed to the intestine via the gallbladder. The small volume of bile in the gallbladder and its inspissated nature suggest a low flux of bile (and therefore of cholesterol carbon) through this route, yet no alterations were observed in circulating levels. There was also no evidence of hepatic insult, at least based on HSI and liver histopathology.

Measurement of SSI was originally included in our design because of the haematopoietic function of the spleen in fish and the reported insult to haematopoiesis in fish by As (NRCC 1978; Oladimeji 1980). An increase in the index (splenomegaly) was postulated, since it would reflect heightened activity of erythrocyte production. No significant effect on this index occurred in the present study. Similarly, there were no arsenite impacts on either hematocrit or hemoglobin. Although other workers have reported reduced hematocrit and/or hemoglobin as a result of exposure to either waterborne (Speyer 1975) or dietary (Oladimeji 1980) arsenite, these studies were less than 8 wk in duration relative to the 17 wk used here. Had an initial alteration occurred during our study, it is possible that the fish adapted by 17 wk, allowing the haematological indicators to return to normal. Adaptation to As has been reported in salmonids (Dixon and Sprague 1981), and, based on the whole-body As concentrations which we report, levels were at their lowest (possibly equilibrium) when the haematological parameters were measured.

Concentrations of three neuroamines (norepinephrine, dopamine, and serotonin) in brain tissue were unaffected by 121 d of arsenite exposure. The response of norepinephrine to stress is variable. Exposure to sodium pentachlorophenate will elicit an increase in whole-brain norepinephrine (Sloley et al. 1986) whereas a reduction in hypothalamic norepinephine of rainbow trout occurs after stressful handling and hypoxia (Mazeaud and Mazeaud 1981). Arsenic can inhibit the norepinephrine-deactivating enzyme monoamine oxidase (MAO) (NRCC 1978) which is present in the teleost brain (Mazeaud and Mazeaud 1981). If MAO activity declined, the concentration of norepinephrine would be expected to rise and a more excitable behaviour would probably result. Although such behaviour was evident at acutely lethal concentrations, neither this behaviour nor the adrenergic response was observed during chronic exposure. The absence of an effect on dopamine, the precursor of norepinephrine, further supports this conclusion. Although significant reductions in brain serotonin concentrations have been associated with convulsive activity by flagfish (*Jordanella floridae*) exposed to methoxychlor (Holdway et al. 1986), neither altered serotonin concentrations nor convulsions were evident here.

The gallbladder lesions were the only internal pathology evident during this study. These lesions suggest that gallbladder function was compromised by both cytological damage and changes in gross structure. The tough thickened inelastic walls and the presence of inspissated bile support the idea that bile storage capacity was reduced. Although it also suggests that bile production itself had been compromised, the absence of hepatic lesions does not support the concept. A similar gallbladder lesion has been noted in rainbow trout exposed to either waterborne or dietary arsenate. McGeachy and Dixon (1990) reported the presence of the lesion after an 11-wk exposure to waterborne arsenate concentrations of 36 $mg \cdot L^{-1}$ at 5°C or 18 $mg \cdot L^{-1}$ at 15°C. Fish exposed to 9 or 18 mg $arsenate \cdot L^{-1}$ (at 15 or 5°C, respectively) over the same period were free of the pathology. Cockell et al. (1991) reported that 71 and 100% of the fish dosed, respectively, with 33 and 65 μg $arsenate \cdot g\ diet^{-1}$ for 24 wk showed chronic inflammatory changes in the subepithelial tissues of the gallbladder wall. The lesion was absent from fish dosed at 13 μg $As \cdot g\ diet^{-1}$. The exact etiology of the gallbladder lesion is unknown. We suspect that the concentrations of As in bile may have been sufficiently high to cause direct uptake to the gallbladder tissue, and hence an inflammatory response. Although we did not measure the As concentration in bile, Penrose (1975) and Sorensen et al. (1979) reported that the highest concentrations of As in fish during exposure are those in bile.

Arsenite uptake during aqueous exposure was somewhat atypical relative to conventional logarithmic assimilation kinetics (Spacie and Hamelink 1985). Arsenic concentrations rose, albeit somewhat erratically, over the first 17 d of exposure. They then decreased, reaching equilibrium after 128 d, a response which suggests increasing excretory efficiency over the exposure period. Evidence for improved excretion efficiency with exposure was reported by Dixon and Sprague (1981) who found that preexposed fish accumulated less As from subsequent exposures than naive fish. Similar results have been reported for rainbow trout during dietary exposure (Oladimeji 1980) and mice during respiratory exposure (Bencko and Symon 1970). Although the mechanism of improved excretion is not fully understood, it most probably reflects the induction or enhancement of the biochemical pathways necessary for the conversion of inorganic As to the organoarsenicals which constitute the major As excretion products. Because information on As speciation in freshwater biota is scarce (Phillips 1990), concensus on the most significant metabolites is lacking. Oladimeji et al.

(1979) suggested that the major organoarsenical in rainbow trout was the ethyl ester of arsenobetaine. Lawrence et al. (1986), however, reported that the organoarsenicals in four divergent freshwater species did not include arsenobetaine, its ethyl ester, or arsenocholine but rather a more hydrophilic but unidentified compound. Cockell (1990) reported that the As in rainbow trout bile after 12 wk of dietary exposure to 58 μg arsenate·g^{-1} diet was 40% arsenite, 29% monomethylarsonic acid, 27% dimethylarsinic acid, and 1% arsenate.

The depuration portion of the kinetics study gave rise to unexpected results. The unusual increases in whole-body As concentration apparent at 12 h were consistent for all treatments and persisted for equivalent time periods. Because of this similar response, plus the fact that procedures for each sampling time were standardized, these anomalies are not considered to be methodological artifacts. Although there is no clear explanation for this transient surge in As content, the stress associated with catching and transferring the fish to As-free water at the start of depuration may have been involved. Increased gill permeability is a well-documented response to stress (Mazeaud and Mazeaud 1981) which permits ions to cross gill epithelium according to the ionic gradient. In freshwater, this ionic gradient normally results in the loss of blood electrolytes to the water. In this case, where ambient As concentrations were high, the blood/water gradient would act to direct As into the fish. The high capacity for the gill to concentrate As by adsorption would also facilitate a net influx by further increasing the As gradient at the gill surface.

Two days after transfer to arsenite-free water, the As concentration in depurating fish had fallen to concentrations that were the same as those observed prior to depuration. The concentrations did not change during the subsequent 10 d of depuration. This suggests that the As which entered during the transitory influx/efflux period at the start of depuration occupied a different and much more kinetically rapid compartment than that taken up (and equilibrated) during the 181-d chronic exposure. Based on these results, we suspect that the As concentrations in fish sampled during field studies could give an unrealistically high estimate of the chronic equilibrated concentration because of netting and capture stress.

In summary, chronic arsenite exposure of juvenile rainbow trout affects growth performance primarily by acting as a limiting factor on available dietary energy. The marginal nature of the growth response is intriguing in that, after taking appetite depression into account, the 9.64 mg·L^{-1} exposure concentration represents a threshold for the bioenergetic effects of arsenite, a response that was only marginally significant even after 18 wk of exposure. Furthermore, this exposure concentration represents a significant proportion (0.52) of the 144-h LC50 and was associated with substantial mortality. Based on our response endpoints, the concentration at which arsenite begins to exert toxicity on rainbow trout lies somewhere between 2.48 and 9.64 mg·L^{-1}. The actual threshold concentration can be estimated, by taking the geometric mean of these limits (Stephan et al. 1985), as 4.88 mg·L^{-1}. We calculated equivalent thresholds for work with rainbow trout (Speyer 1975) and fathead minnow (*Pimephales promelas*) (Lima et al. 1984) to be 4.24 and 3.03 mg arsenite·L^{-1}, respectively. At 4.05 (23%) mg·L^{-1}, the mean (CV) of the three estimates is remarkably consistent considering the divergent nature of the species.

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Pelagic Food Chain Structure in Ontario Lakes: A Determinant of Mercury Levels in Lake Trout (*Salvelinus namaycush*)

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The trophic structure of pelagic communities in glaciated regions is highly variable due to restricted dispersal of glacial relict taxa and recent species introduction. Much of the between-lake variation in Hg in lake trout (*Salvelinus namaycush*) flesh from the St. Lawrence system (non-point-source contaminated lakes), which spans more than two orders of magnitude (0.03–3.96 μg/g), results from differences in the length of pelagic food chains. Hg levels from the longest food chains where pelagic forage fish and the crustacean *Mysis relicta* were present were about 3.6-fold higher than those from the shortest food chains where these last two trophic components were missing. A lack of correlation between Hg levels in smallmouth bass (*Micropterus dolomieu*), which do not exploit the pelagic food chain, and the length of the pelagic food chain confirmed that the positive relationship observed in lake trout could not be attributed to the confounding effect of some physicochemical factors covarying with our pelagic food chain classification. Our results confirmed that the food chain biomagnification model developed by Rasmussen et al. (1990. Can. J. Fish. Aquat. Sci. 47: 2030–2038) to predict PCB levels in lake trout can also be applied to Hg biomagnification.

La structure trophique des communautés pélagiques dans les régions de glaciation est extrêmement variable à cause de la dispersion restreinte de taxons reliques de l'ère glaciaire et de l'introduction d'espèces récentes. Une bonne partie de la variation entre les lacs de la concentration de Hg dans la chair du touladi (*Salvelinus namaycush*) dans le réseau du Saint-Laurent (lacs qui ne sont pas soumis à des sources ponctuelles de pollution), qui couvre plus de deux ordres de grandeur (0,03–3,96 μg/g) provient de différences dans la longueur des chaînes trophiques pélagiques. Les concentrations de Hg dans les chaînes les plus longues, où étaient présents des poissons fourrages pélagiques et le crustacé *Mysis relicta*, étaient environ 3,6 fois plus élevées que celles des chaînes plus courtes où manquaient au moins deux composantes trophiques. L'absence de corrélation entre les teneurs de Hg de l'achigan à petite bouche (*Micropterus dolomieu*), qui n'exploite pas la chaîne trophique pélagique, et la longueur de la chaîne pélagique confirme que la relation positive observée chez le touladi ne peut être attribuée à l'effet trompeur de certains facteurs physico-chimiques qui varient en même temps que notre classification de la chaîne trophique pélagique. Notre résultat a confirmé que le modèle de bioamplification de la chaîne trophique mis au point par Rasmussen *et al.* (1990. J. can. sci. halieut. aquat. 47 : 2030–2038) pour prédire les concentrations de BPC chez le touladi peuvent aussi s'appliquer à la bioamplification du Hg.

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Uptake from food and water have been shown to be two complementary routes through which contaminants such as heavy metals and organic chemicals accumulate in aquatic organisms (reviewed by Biddinger and Gloss 1984). While bioconcentration from water appears to be the predominant pathway for bioaccumulation of a large number of chemicals (Biddinger and Gloss 1984), uptake from food has also been shown to be important for a number of persistent compounds (e.g., DDT: Macek and Korn 1970; PCBs: Thomann 1981; Sodergren 1984; selenium; Sandholm et al. 1973; Bertram and Brooks 1986; Hg: Boudou et al. 1979). The bioaccumulation of these often toxic substances from food implies that they become biomagnified along the food chain, reaching high concentrations in top consumers such as large predators, including man. While this popular concept is by no means recent (Woodwell et al. 1967; Macek and Korn 1970), some problems have so far limited its role in the development of a predictive theory of the ecological fate of biomagnifying contaminants (but see Thomann 1981).

For example, predators generally have higher contaminant concentrations than their potential prey, as predicted

by the biomagnification hypothesis (e.g., PCBs: Niethammer et al. 1984; Hg: Ratkowsy et al. 1975; Philips et al. 1980; Akielaszek and Haines 1981; Wren et al. 1983). However, it has been suggested that other confounding factors, such as differences in size, age, or metabolic rate between predator and prey, may well be causing this apparent biomagnification (Moriarty 1975; Biddinger and Gloss 1984).

A further complication resides in the inherent difficulty in defining concepts such as "food chain" and "trophic level" in measurable terms (Peters 1991). This last problem becomes more apparent when one is concerned with intraspecific variation in trophic level rather then interspecific predator/prey comparisons. For example, while it can be safely argued that adult pike and zooplankton differ in trophic level, it is much more difficult to establish that pike in one lake occupy a trophic level higher than in another lake.

Rasmussen et al. (1990) attempted to overcome these problems in their study of PCB concentration in lake trout (*Salvelinus namaycush*) from 81 Canadian Precambrian Shield lakes. They showed that intraspecific biogeographical variation in the length of the food chain leading to these pelagic predators determined their white muscle PCB concentration. Trophic level of lake trout was operationally defined by the presence or absence of intermediate species (the crustacean *Mysis relicta* and pelagic forage fish such as smelt and coregonids) trophically linking lake trout to small zooplankton. PCB concentrations were found to approximately triple with each additional intermediate trophic step (from lakes free of *Mysis* and pelagic forage fish, through *Mysis*-free lakes containing pelagic forage fish, to lakes containing both *Mysis* and pelagic forage fish).

In principle, this predictive model of the fate of PCBs in lake communities based on a simplified description of the pelagic food chain should be applicable to other potentially biomagnifying persistent chemicals. Here, we test the Rasmussen et al. (1990) model using Hg concentrations in lake trout from Ontario lakes not subjected to point-source contamination. We predict that Hg concentration in lake trout should parallel that of PCBs, increasing from lakes where lake trout are mostly limited to eating zooplankton to lakes with the longest food chains (lakes containing both *Mysis* and pelagic forage fish).

Smallmouth bass (*Micropterus dolomieu*) can be found in the three trophic classes of lakes we investigated and is a predominantly littoral species feeding mainly on crayfish and cyprinids (Scott and Crossman 1973). Being essentially "disconnected" from the pelagic food chain, smallmouth bass represent a good control for the effect of some physicochemical variable(s) influencing Hg levels in fish independently of food chain length. Lack of a relationship between the length of the pelagic food chain and Hg in smallmouth bass, in conjunction with a positive relationship in lake trout, would, therefore, further support the importance of food chain biomagnification per se in explaining variation in Hg in fish. It would also weaken the possibility that a positive relationship between Hg concentration and trophic level in lake trout could be attributed to the spurious effect of some lake characteristic(s) covarying with the length of the pelagic food chain.

Physicochemical variables related to lake morphometry (maximum depth: Wren and McCrimmon 1983; lake area: Wren et al. 1991) and water chemistry (e.g., pH: Wren and McCrimmon 1983; Suns and Hitchins 1990; McMurtry et al. 1989; Wren et al. 1991; calcium: Wren et al. 1991; alkalinity: Swain and Helwig 1989) have been shown to influence Hg levels in freshwater fish. Such factors could either obscure trophic level effects on Hg in fish or create a spurious relationship between trophic level and Hg concentration in fish by covarying with fish trophic level. Here, we examined potential effects of lake area and maximum depth, Secchi depth, pH, and conductivity on Hg concentration in lake trout and smallmouth bass.

Methods

The fish Hg database was obtained from the Sports Fish Contaminants Monitoring Program of the Water Resources Branch, Ontario Ministry of the Environment (MOE). Total Hg was analyzed by flameless atomic absorbtion spectrophotometry with a minimun detection limit of 0.01 ppm (MOE 1981). Recent work (Grieb et al. 1990) has shown that in fish, practically all Hg is in the form of methyl mercury and therefore, total Hg essentially measures methyl mercury. Fish were homogenized followed by oxidation with hydrogen peroxide and digestion with a solution of 2:1 sulfuric acid at 60°C. Further technical details pertaining to sample handling are in MOE (1981). We set an arbitrary minimum sample size of 10 individuals before including any species in the study. Fish Hg measurements were reported over the period 1975–84 and data were pooled in the few cases where information from more than one year was available.

Data on lake morphometry and physicochemical variables were obtained from the computerized data inventory of the Ontario Ministry of Natural Resources (MNR), the Ontario Acid Sensitivity Database (MOE) (Neary et al. 1990), and Dadswell (1974).

The food chain classification was based on the presence or absence of pelagic forage fish species (smelt, ciscoes, lake and round whitefish, alewife, and ninespine stickleback) as determined from a published database (MNR). The presence or absence of *Mysis* was determined from Dadswell's (1974) extensive monograph. In those lakes not studied by Dadswell, the presence/absence of *Mysis* was determined from a map showing the maximum extent of glacial lakes (Douglas 1970). Dadswell found *Mysis* to be absent from only about 10% of lakes situated within the glacial-lake boundaries in Ontario. Thus, there is a slight chance of classification error among the lakes classified as *Mysis* positive on the basis of glacial lake maps. He also never found *Mysis* in southern Ontario lakes shallower than 18 m (maximum depth), and we accordingly classified such lakes as *Mysis* free.

Following Rasmussen et al. (1990), lakes were classified according to the number of intermediate trophic levels linking zooplankton to lake trout. These intermediates include the opossum shrimp *M. relicta* and pelagic fish such as coregonids (*C. artedi* and *C. rotundiformis*), rainbow smelt, alewife, and ninespine stickleback. We refer to lakes without *Mysis* or pelagic forage fish as Class 1, lakes with pelagic forage fish but no *Mysis* as Class 2, and lakes with both *Mysis* and pelagic forage fish as Class 3. While we do not claim to know the exact trophic relationships within the pelagic communities of individual lakes, a number of studies on feeding relationships in lakes have supported this classification. Studies on adult lake trout from Class 3 lakes

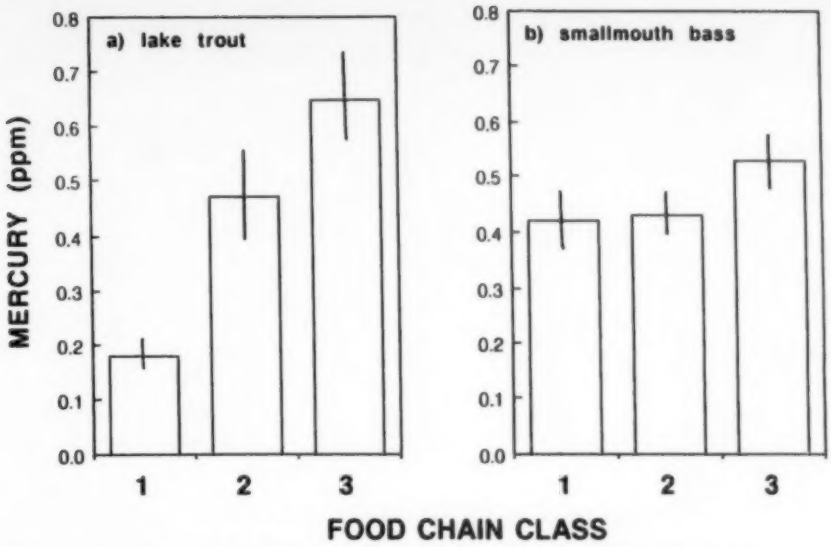

FIG. 1. Geometric mean (±1 SE) concentration in the muscle (a) lake trout and (b) smallmouth bass in relation to the trophic structure of the pelagic community. Class 1 lakes contain no *Mysis* or pelagic forage fish; Class 2 lakes contain pelagic forage fish but no *Mysis*; Class 3 lakes contain both *Mysis* and pelagic forage fish.

have shown them to be piscivorous, feeding mainly on smelt, ciscoes, alewife, and whitefish (Dymond 1928, Rawson 1930, 1951, 1961; Van Oosten and Deason 1937; Dryer et al. 1965; Scott and Crossman 1973; McCrimmon et al. 1983). The pelagic forage fish eaten by lake trout in these lakes (mainly smelt, coregonids, and alewife) feed heavily on *Mysis* (Rawson 1951; Wells 1968; Scott and Crossman 1973), and *Mysis* prey on zooplankton and some large phytoplankton (Carpenter et al. 1974; Martinez and Bergersen 1989). When *Mysis* is absent (Class 2 lakes), the pelagic forage fish utilize mostly zooplankton (Price 1963).

If both *Mysis* and pelagic forage fish are absent (Class 1 lakes), lake trout feed exclusively on zooplankton and benthic invertebrates for a large part of the year because for most of their growing season, they are isolated from inshore forage fish by a thermal barrier (Martin 1954, 1966; Monroe and Hicks 1984). We hypothesized that if variation in food chain length is responsible for much of the variation in lake trout Hg levels, the lowest Hg concentration should be found where *Mysis* and pelagic forage fish are absent (Class 1) and the highest levels where both trophic links are present (Class 3).

In contrast with lake trout, smallmouth bass is a littoral species that feeds very little, if at all, on pelagic coregonids and smelt (Doan 1940; Westman and Westman 1949; Emery 1975). We therefore used smallmouth bass as a control for the potential confounding effect of some physicochemical factors influencing Hg levels in fish that could also fortuitously covary with the length of the pelagic food chain. While we did not expect to see an effect of pelagic food chain structure on Hg levels in bass, we did expect to find some correspondence between Hg levels in lake trout and bass, reflecting the extent to which the two species are influenced by lake-specific variation in the overall exposure to Hg. Thus, this two-species analysis will allow us to, in effect, partition the variation in lake trout Hg level explained by food chain structure from the variation associated with lake-specific contamination factors, even though the latter factors are not yet explicitly modelled.

All statistical analyses were based on lake averages of mercury concentration and fish weight. We investigated the effects fish weight, trophic level, and physicochemical variables (such as area and maximum depth, pH, conductivity, and Secchi depth) on Hg in lake trout and smallmouth bass through multiple regression and analysis of variance (ANOVA) (Draper and Smith 1981). Logarithmic transformations were used for all these variables (except pH) to normalize the data and reduce heteroscedasticity. The statistical significance of each parameter in a multiple regression model was tested using *t*-tests.

Results

Hg levels in Ontario lake trout increased with the length of the pelagic food chain (ANOVA, $P < 0.001$) (Table 1; Fig. 1a). Trout from lakes with the shortest food chains (Class 1 lakes) had the lowest Hg levels (geometric mean Hg = 0.18 μg/g), followed by lakes with food chains of intermediate length (Class 2 lakes; geometric mean Hg = 0.47 μg/g). Trout from lakes with the longest food chains (Class 3 lakes) had the highest Hg levels (geometric mean Hg = 0.65 μg/g). The relative increase in Hg levels attributed to the presence of forage fish in the pelagic food chain (Class 2/Class 1: 2.6-fold biomagnification factor) was therefore about twice as important as that attributed to the presence of *Mysis* in food chains containing pelagic forage fish (Class 3/Class 2: 1.4-fold biomagnification).

Multiple regression showed that three variables, average body weight, food chain class, and maximum lake depth, were found to explain a significant (*t*-test, $P < 0.05$) proportion of the total variance in Hg in lake trout. Other variables tested were not significant when subsequently added and

TABLE 1. Hg content of lake trout and smallmouth bass from Ontario Lakes with different pelagic communities. For each lake, the following physicochemical variables are listed: latitude (lat), longitude (long), lake area, maximum depth (Z_{max}), pH, conductivity (cond.), and Secchi depth.

Lake trout												
Lake	Lat.	Long.	Area (ha)	Z_{max} (m)	pH	Cond. (μS/cm)	Secchi (m)	*Mysis*[a]	Forage fish[b]	Weight (g)	Hg (μg/g)	*n*
*Class 1 (*Mysis *and pelagic forage fish absent)*												
Bear	45°20′	78°42′	95	36.6	6.1	34	7.9	–*	–	402	0.28	22
Beaver	46°57′	80°51′			6.9	58		–*	–	109	0.05	49
Big Porcupine	45°27′	78°37′	235	31.7	6.0	27	7.6	–*	–	410	0.18	24
Blue	49°10′	81°15′						–*	–	744	0.06	14
Blue Chalk	45°12′	78°56′	50	22.0	6.9	29	6.4	–*	–	1309	0.24	19
Bluesucker	47°10′	80°37′	144	21.4	5.1	36	8.8	–*	–	1275	0.23	18
Bonnechere	45°28′	78°35′	104	22.0	5.6	27	6.7	–*	–	542	0.33	26
Camp	45°26′	78°55′	189	42.7	6.2	32	6.2	–	–	396	0.15	21
Canisbay	45°34′	78°35′	152	25.9	6.3	34	6.4	–*	–	1440	0.64	11
Centre	45°01′	78°03′	144	17.7			3.1	–*	–	1325	0.49	10
Eels	44°54′	78°08′	945	29.9	6.7		3.3	–	–	488	0.09	13
Fletcher	45°21′	78°47′	256	23.2	6.1	34	3.9	–*	–	860	0.25	21
Iron	47°04′	79°55′	72	27.0	5.7	42	10.3	–*	–	426	0.03	44
Kimball	45°21′	78°41′	212	67.1	6.3	30	9.1	–*	–	690	0.32	36
Kukagami	46°44′	80°33′			5.8	59		–*	–	779	0.05	24
Laundrie	47°07′	80°52′	374	20.4	5.2	33	9.3	–*	–	324	0.14	109
Livingstone	45°22′	78°43′	189	36.6	6.5	37	4.6	–	–	1392	0.38	22
Louisa	45°28′	78°29′	490	61.0	6.1	32	7.7	–	–	540	0.44	30
McCraney	45°34′	78°54′	361	61.3				–*	–	505	0.35	16
McFadden	45°20′	78°51′	54	30.5	6.5	37	7.2	–*	–	901	0.18	22
McGarvey	45°26′	78°34′	70	20.0	5.9	23	3.6	–*	–	2533	0.35	12
Miskwabi	45°03′	78°19′	264	44.2				–*	–	867	0.13	20
Nunikani	45°12′	78°44′	116	24.1	6.2	29	7.2	–*	–	670	0.17	23
Pedro	46°55′	80°32′	64	11.0	5.9	39	5.3	–*	–	782	0.03	58
Percy	45°12′	78°22′	596		7.4	47	3.0	–*	–	502	0.39	14
Pilgrim	47°11′	80°41′	123	25.0	5.2	32	8.0	–*	–	383	0.06	38
Red Chalk	45°11′	78°57′	58	32.0		30	6.1	–*	–	1195	0.22	20
RedPine	45°12′	78°42′	380	41.5	6.4	32	7.5	–*	–	805	0.18	18
Regan	47°14′	80°50′	122	38.0	6.2	43		–*	–	463	0.04	36
Round Island	45°44′	78°12′	156	17.0	6.4	35	4.5	–*	–	973	0.54	15
Saymo	46°59′	83°31′			6.7	38		–*	–	833	0.39	13
Seagram	47°06′	80°32′	102	19.0	5.1	36	6.5	–*	–	706	0.15	15
Silent	44°55′	78°04′	115	23.1	6.9	33	5.4	–*	–	1018	0.24	15
Slipper	45°17′	78°42′		34.0	6.4	28		–*	–	387	0.15	20
St.-Peter	45°19′	78°02′						–*	–	858	0.43	17
Geometric mean										686.1	0.18	
Class 2 (no Mysis*, pelagic forage fish present)*												
Bark	45°27′	77°51′	3792	87.5	6.6	45		–	W, S	2788	1.53	30
Bella	45°26′	79°02′	328	40.0	6.8		3.3	–	W, S	3532	0.55	31
Bending	49°19′	92°08′	1137	45.8	7.0	29	4.6	–*	W, C	1565	0.67	20
Caribou	45°56′	80°04′	529	59.5				–	C	1488	0.51	17
Carson/Stubb	45°30′	77°45′						–	W	1636	0.57	15
Como	47°55′	83°30′	1596	26.0	7.0	76	4.7	–*	SB	3797	1.29	15
Drag	45°04′	78°23′	912	55.0	6.9	73	5.5	–	C, W	367	0.14	11
Fernow	49°55′	86°05′	688	38.0				–*	S, W, P	1364	0.39	21
Greenwater	48°34′	90°26′	3340	54.9	7.7	64	4.4	–*	C, W	1216	0.34	24
Harp	45°23′	79°07′	72	33.0	6.7	35	3.7	–	C	621	0.88	18
Kamaniskeg	45°25′	77°41′	2909	40.0	6.7	50		–	S, W, C	3613	0.96	10
Kawagama	45°18′	78°45′	2819	73.2	6.5		8.4	–	C, W	485	0.17	29
Koshlong	44°58′	78°29′	401	42.7	6.3	36	7.0	–	S, C	1818	0.91	30
Lamuir	45°50′	78°35′	757	39.3				–*	W	2028	0.50	11
Lavieille	45°51′	78°14′	2428	48.8	6.6	48	4.7	–	C, W	2273	1.04	11
Limerick	44°53′	77°37′	829	30.0	8.5		5.3	–	RW	1922	0.63	19
Littlehawk	45°09′	78°43′	343	93.0	6.2	32	6.9	–	C, SB	931	0.33	15
McKay	49°37′	86°25′	139	48.8	5.6	26	3.4	–*	C, W	1234	0.41	16

TABLE 1. (*Continued*)

Lake	Lat.	Long.	Area (ha)	Z_{max} (m)	pH	Cond. (μS/cm)	Secchi (m)	*Mysis*[a]	Forage fish[b]	Weight (g)	Hg (μg/g)	*n*
Lake trout												
Opeongo	45°42′	78°23′	5158	49.4	6.5	43	5.5	−	W, C	1107	0.50	16
Oxtongue	45°22′	78°55′	250	26.8	6.2	35	4.4	−	S, W, C	2812	1.61	19
Papineau	45°21′	77°49′	792	61.0	6.5	94	5.6	−	C, W	583	0.51	11
Peshu	46°58′	83°08′	389	50.6	7.3	43	6.1	−*	W	72	0.16	13
Pedstone	45°11′	78°42′	1194	82.4	6.4	38	8.1	−	W	567	0.24	16
Round	45°38′	77°30′	3069	54.9	7.7	66		−*	W	2578	1.40	39
Shakwa	46°46′	81°59′	649	27.5	6.5	33	5.9	−*	W	75	0.04	41
Sherborne	45°11′	78°47′	252	35.1	5.7	31	9.2	−	S	5907.7	0.19	20
Smoke	45°31′	78°41′	608	54.9	6.5	35	6.7	−	RW	1011.1	0.30	41
Treeby	47°52′	84°51′	130		6.9	73	3.8	−*	C, W	1498.7	0.76	10
Geometric mean										1213.2	0.47	
*Class 3 (*Mysis *and pelagic forage fish present)*												
Aegean	50°49′	94°48′	482	59.0	6.7	25	4.8	+*	W	1913	1.32	18
Bear	46°12′	81°27′	683	36.6	7.0	60	8.3	+*	C	2001	0.23	16
Bernard	45°45′	79°23′	2058	47.9	6.7		3.9	+	S, W	2423	0.67	32
Boshkung	45°04′	78°44′	716	71.1	7.1		6.0	+	S, W, C	1161	0.44	20
Charleston	44°32′	76°00′	2519	91.5	8.5		3.7	+	A, W, C	966	0.36	15
Delaney	50°05′	94°03′	1282	81.4				+*	W	1880	0.27	31
Eden	50°40′	94°59′	489	24.6				+*	C, W	1343	0.33	39
Fairy	45°20′	79°11′	712	69.5	6.5		3.6	+	S, W, C	4163	2.68	28
Gillies	45°12′	81°21′	228	34.0			8.3	+*	C, W	170	0.17	16
Gooseneck	50°02′	94°48′	172	29.9			4.3	+*	C	2126	0.73	31
Hawley	54°30′	84°39′	1235	54.0				+*	C, W, P	1081	0.38	15
Joseph	45°10′	79°44′	4268	90.0	6.7		9.6	+	S, W	2205	0.35	25
Kioshkokwi	46°05′	78°53′	1127	45.8				+	C, W	1173	1.25	10
Lake of Bays	45°15′	79°04′	6105	53.0	6.5		4.2	+	S, W, C	3280	1.07	58
Larder	48°05′	79°38′	3704	33.5				+	C, W	1351	0.35	10
Loughborough	44°27′	76°25′	1066	34.0			4.8	+	C, W, S, A	2821	0.36	26
Mazinaw	44°50′	77°12′	1572	135.0	7.3		6.0	+	C, W	1591	1.22	21
McCarroll	46°26′	83°56′	198	33.6	7.1	45	5.0	+*	C	1242	0.56	12
Miskokway	45°39′	80°14′	238	41.2				+*	C	787	0.72	28
Muskoka	45°00′	79°30′	12215	66.5	6.7	46	4.9	+	C, W, S	2947	1.97	83
Muskrat	45°40′	76°55′	1202	64.0	8.4		1.5	+	S	2617	0.58	19
Quetico	48°34′	91°55′	4265	61.0	6.9	23	3.4	+*	C, W	1632	0.67	17
Raven	48°03′	79°33′	617	46.9	6.9		2.9	+	W	1381	0.62	12
Rosseau	45°10′	79°35′	6374	90.0				+	C, W, S	2027	0.86	44
Snook	50°12′	94°41′	263	37.2	6.7	33	1.8	+*	C	1316	0.80	71
Sutton	54°15′	84°44′	3764	82.0				+*	C, W, P	1403	1.00	15
Trout	45°35′	80°10′	290	37.2	6.0	28	2.4	+*	C	1349	0.36	21
Trout	46°18′	79°20′	1675	69.2	7.1	78	3.8	+	S, W, RW, C	5331	0.96	22
Trout	46°13′	80°35′	930	36.6	6.6	45	3.2	+	C	1932	0.55	11
Trout	50°14′	94°54′	110	34.8			4.6	+*	C	2152	0.65	16
Twelve Mile	45°01′	78°43′	337	27.5	6.8	52	4.1	+	S	740	0.36	20
Vernon	45°20′	79°17′	1506	37.5	6.1		3.1	+	S, W	3129	3.94	36
Wilson	50°30′	95°02′	312	42.7			1.5	+*	C, W	912	0.90	13
Geometric mean										1614.9	0.65	

were dropped from the regression model. Even though there was a large amount of overlap in maximum depth between the three classes of lakes (Table 1), there was a significant increase in maximum depth from Class 1 to Class 3 lakes (ANOVA, $P < 0.001$). This raised the possibility that the significant positive regression coefficient between Hg and maximum depth could simply be the result of a collinearity between the length of the pelagic food chain and maximum depth, as was observed by Dadswell (1974). Alternatively, maximum depth could truly represent the effect of some physicochemical factor(s) influencing Hg concentration in lake trout, independent of the trophic structure of the lake. However, even though maximum depth varied greatly within food chain class (Table 1), it was not retained in the multiple regression when the analysis was performed within food chain classes (*t*-test, Class 1: $P > 0.10$; Class 2: $P > 0.45$; Class 3: $P > 0.14$). This shows that the significant regression coefficient of maximum depth in the multiple regression model was due to a collinearity with the length of the pelagic food chain rather than an independent effect of maximum

TABLE 1. (*Continued*)

Lake	Lat.	Long.	Area (ha)	Z_{max} (m)	pH	Cond. (μS/cm)	Secchi (m)	*Mysis*[a]	Forage fish[b]	Weight (g)	Hg (μg/g)	*n*
						Smallmouth bass						
						*Class 1 (*Mysis *and pelagic forage fish absent)*						
Bear	45°20′	78°42′	95	36.6	6.1	34.0	7.9	−*	−	615	0.48	18
Centre	45°01′	78°03′	144	17.7			3.9	−*	−	282	0.71	14
Kimball	45°21′	78°41′	213	67.1	6.3	30.0	9.1	−*	−	472	0.72	29
Lower Hay	45°24′	78°12′	420	32.3			1.9	−*	−	216	0.38	10
McKenzie	45°22′	78°01′	312	27.8	6.5	46.0	3.8	−*	−	363	0.44	16
Miskwabi	45°03′	78°19′						−*	−	402	0.35	17
Nunikami	45°12′	78°44′	116	24.1	6.2	29.0	7.2	−*	−	88	0.23	37
Redpine	45°12′	78°42′	380	38.7	7.3	32.0	7.5	−	−	285	0.28	23
Silent	44°55′	78°04′	115	23.1	6.9	33.0	5.4	−*	−	357	0.41	15
Geometric mean										305.2	0.42	
						Class 2 (no Mysis*, pelagic forage fish present)*						
Baptiste	45°07′	78°03′	1952	31.0				−	W, C	903	0.84	12
Bella	45°26′	79°02′	337	35.0	6.8		3.3	−	W, S	745	0.37	20
Calabogie	45°16′	76°45′	1316	31.1	7.7			−*	W	601	0.59	39
Eagle	45°50′	79°30′	991	22.0	6.4	36.0	5.0	−	W, S	800	0.33	18
Galeairy	45°30′	78°17′	889	22.9	6.4	36.0	4.3	−	W	222	0.41	16
Jack	44°42′	78°02′	1237	51.2			3.6	−	C	678	0.40	21
Kamaniskeg	45°25′	77°41′	2909	40.0	6.7	50.0		−	S, W, C	264	0.25	11
Kashagawigamog	44°59′	78°36′	818	39.7	7.1	79.0	5.2	−*	W, C	391	0.24	16
Koshlong	44°58′	78°29′	401	42.7	6.3	36.0	7.0	−	S, C	1003	0.91	12
Limerick	44°53′	77°37′	744	29.0			5.3	−	RW	452	0.52	20
Littlehawk	45°09′	78°43′	343	86.9	6.2	32.0	6.9	−	C, SB	705	0.56	20
Opeongo	45°42′	78°23′	6000	45.0	6.5		3.6	−	W, C	325	0.37	46
Oxtongue	45°22′	78°55′	246	27.0	6.1		2.2	−	S, W, C	266	0.50	32
Paudash	44°58′	78°03′	755	46.4	7.0	110.0	5.2	−	C	525	0.36	22
Smoke	45°31′	78°41′	607	54.9	6.5	35.0	6.7	−	RW	293	0.31	11
Sydemham	44°25′	76°33′	451	36.6			3.7	−	C	439	0.42	10
Windermere	47°58′	83°47′	3832	30.0			4.8	−*	W	1059	0.37	11
Geometric mean										507.8	0.43	
						*Class 3 (*Mysis *and pelagic forage fish present)*						
Bay	45°30′	79°12′	133	31.1	6.5		6.2	+	W, S	592	0.61	21
Bernard	45°45′	79°23′	2058	47.9	6.7		3.9	+	S, W	403	0.41	20
Boshkung	45°04′	78°44′	716	71.1	7.1		6.0	+	S, W, C	784	0.50	16
Brandy	45°06′	79°31′	105	7.5				+*	C	421	0.93	10
Caribou	45°56′	80°04′	529	59.5				+*	C	615	0.48	19
Cecebe	45°38′	79°33′	770	19.5				+*	W, C, S	613	0.59	19
Delaney	50°05′	94°03′	1282	81.4				+*	S	668	0.27	24
Fairy	45°20′	79°11′	667	74.0	6.5		3.6	+	S, W, C	353	1.14	40
Falls	46°17′	83°02′	169	10.1				+*	W, C, S	293	0.29	15
Gloucester Pool	44°51′	79°42′	1378	30.5	7.9			+	W, C, A	1139	0.57	13
Gooseneck	50°02′	94°48′	172	29.9			4.3	+*	C	983	0.96	40
Haines	45°21′	79°56′	112	19.2	6.1	45.0	3.2	+*	C	130	0.57	12
Joseph	45°10′	79°44′	4268	90.0	6.7		9.6	+	S, W	1015	0.58	21
Kagawong	45°49′	82°18′	5556	33.5				+*	S	395	0.39	15
Lake of Bays	45°15′	79°04′	6105	53.0	6.5		4.2	+	S, W, C	940	0.80	33
Lang	46°10′	81°40′	597	21.0	7.2	70.4	6.3	+	W, C	161	0.30	15
Mackie	45°05′	76°59′	157	22.9				+*	W	391	0.32	14
Mazinaw	44°50′	77°12′	1572	135.0				+	C, W	972	0.78	12
Mill	45°22′	80°00′	619	40.9	7.0		2.1	+*	W, C	459	0.80	16
Miskokway	45°39′	80°14′	238	41.2				+*	C	152	0.14	24
Muskoka	45°00′	79°25′	12215	66.5	6.7	46.0	4.9	+	C, W, S	426	0.99	40
Oliver	45°31′	79°48′	52	15.0				+*	C	414	0.31	14
Peninsula	45°20′	79°11′	650	33.0	6.8		5.2	+	S	539	0.46	31
Rainy	45°32′	79°30′	259	9.2				+*	C	321	0.49	20
Rousseau	45°10′	79°35′	6374	90.0				+	C, W, S	822	0.78	15

TABLE 1. (*Concluded*)

			Smallmouth bass									
Lake	Lat.	Long.	Area (ha)	Z_{max} (m)	pH	Cond. (μS/cm)	Secchi (m)	*Mysis*[a]	Forage fish[b]	Weight (g)	Hg (μg/g)	*n*
Tomiko	46°32′	79°49′	1542	24.0	6.1		3.3	+	W	547	0.94	16
Trout	46°13′	80°35′	930	36.6	6.6	45.0	3.2	+	C	647	0.37	15
Twelve Mile	45°01′	78°43′	337	27.5	6.8	52.0	4.1	+	S	639	0.30	20
Vernon	45°20′	79°17′	1506	37.5	6.1		3.3	+	S, W	364	0.98	49
Geometric mean										489.2	0.53	

[a]Presence or absence for lakes marked with an asterisk was predicted from the distribution of proglacial lake sediments.
[b]A = alewife (*Alosa pseudoharengus*); C = lake cisco (*Coregonus artedi*); W = lake whitefish (*Coregonus clupeaformis*); RW = round whitefish (*Prosopium cylindraceum*); S = rainbow smelt (*Osmerus mordax*); P = ninespine stickleback (*Pungitius pungitius*).

depth on Hg bioaccumulation. Removing maximum depth from the multiple regression, the final model for lake trout was

(1) log Hg (μg/g wet) = −2.72 + 0.21 (±0.04) no. of trophic levels (1, 2, or 3) + 0.61 log weight (g) (±0.09);

$$SE_{est} = 0.28,\ R^2 = 0.56,\ n = 96.$$

Both predictor variables were highly significant (*t*-test, $P < 0.0001$). The number of trophic levels represents the lake Class number, i.e., the number of trophic levels above zooplankton.

Hg levels in smallmouth bass were not related to the length of the pelagic food chain ($r = 0.22$, $n = 55$, $P > 0.05$) (Fig. 1b). Power analysis (Cohen 1969, table 3.3.5) showed that the probability of wrongly accepting the null hypothesis of no effect of food chain class on Hg concentration in smallmouth bass (type II error), when the true correlation between these two variables would have been the same as that found in lake trout ($r = 0.59$), was less than 1%. The lack of a relationship between Hg concentration in smallmouth bass and the classification of the pelagic food chain cannot therefore be ascribed to the somewhat smaller number of lakes in the bass data set. The best model to predict Hg concentration in smallmouth bass included only body weight ($P < 0.05$); the other morphometric and chemical variables did not enter into the model ($P > 0.05$):

(2) log Hg (μg/g wet) = −2.50 + 0.65 log weight (g) (±0.08); $SE_{est} = 0.33$, $R^2 = 0.34$, $n = 55$.

Discussion

Our results support previous observations that the Hg concentration in top predators such as lake trout and northern pike (*Esox lucius*) is greatly influenced by the presence or absence of certain species representing important trophic links between these species and zooplankton (Akielaszek and Haines 1981; Bjorklund et al. 1984; Meili 1991; Rask and Metala 1991). This study extends that of Rasmussen et al. (1990) by showing that a simple food chain classification based on the presence/absence of well-known pelagic taxa can be used to predict the concentration in fish of two chemically unrelated compounds, Hg and PCBs. Indeed, PCB levels in lake trout obtained from Rasmussen et al. (1990) were significantly correlated ($P < 0.0001$) with Hg levels in the lake trout in the lakes for which information about both contaminants is available (Fig. 2). Patterns observed

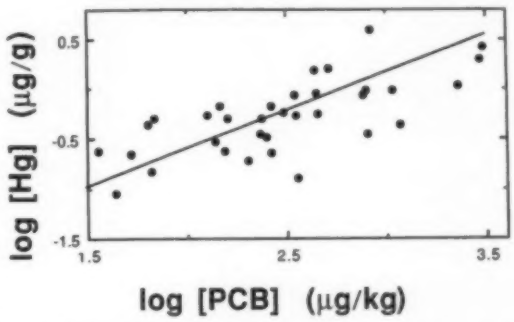

FIG. 2. Relationship between the levels of Hg in the muscle and the levels of PCBs (wet weight) in lake trout in 34 Ontario lakes. PCB data were obtained from table 1 in Rasmussen et al. (1990); Hg data are from this study (Table 1). The geometric mean regression line is shown.

in the distribution of Hg in this study paralleled those observed by Rasmussen et al. (1990) for PCBs in similar communities: contaminant concentration in pelagic species, such as lake trout, was related to the length of the pelagic food chain and contaminant concentration in littoral species, such as smallmouth bass, could not be predicted from the length of the pelagic food chain. Taken together, these two results suggest that pelagic food chains in these Ontario lakes are essentially disconnected from litoral food chains. This has several important consequences for our understanding and prediction of the distribution of biomagnifying compounds in freshwater lakes.

Contaminant concentration in species positioned in different, disconnected food chains within the same lake will show little or no covariation across lakes. For example, Wren et al. (1991) found a statistically significant but only moderate correlation ($r^2 = 0.60$) between Hg in pike and walleye (*Stizostedion vitreum*) in 79 Ontario lakes. While these species usually feed on similar prey (Scott and Crossman 1973), their diets diverge considerably in some lakes. For example, in some lakes, walleye, but not pike, feed extensively on pelagic fish such as smelt (Mathers and Johansen 1985), and the relatively large amount of between-lake variation in walleye Hg concentration (40%), unexplained by Hg concentration in pike, might be due to between-lake variation in the walleye's position along the littoral/pelagic gradient. Species more trophically isolated, as in the case of lake trout and smallmouth bass, will be

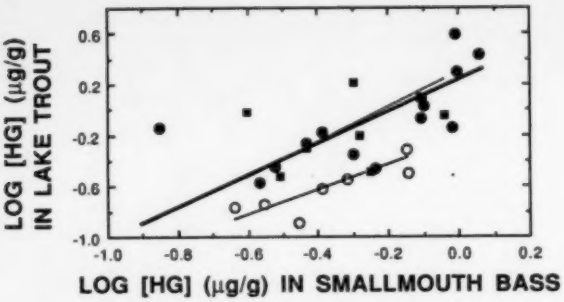

FIG. 3. Relationship between Hg levels in lake trout and smallmouth bass in 29 Ontario lakes. Class 1, 2, and 3 lakes are represented by open circles, solid squares, and solid circles, respectively. The geometric regression lines for each lake trout trophic level class are shown (*t*-test, Class 1 (fine solid line): $P < 0.01$; Class 2 (broken line): $P < 0.05$; Class 3 (bold lines): $P < 0.001$).

expected to show even weaker correlations. Indeed, Hg levels in these two species in lakes where both species were represented in our data set were poorly (but significantly, $P < 0.01$) correlated with each other ($r^2 = 0.34$, $n = 29$) (Fig. 3). This correlation was essentially unchanged ($r^2 = 0.31$, relationship not shown) when the residuals from the Hg/body weight relationships were used instead of the simple mean Hg levels. An analysis of covariance including Hg in bass as a covariate and lake trout trophic class as an effect accounted for 61% of the total variation in Hg in lake trout. This increase in predictive power from the simple two-species relationship indicates that the effect of variation in a common environment, which is reflected by the relationships between the two species within trophic level class, is approximately equal to the effect of trophic level variation in lake trout. Thus, food chain effects on Hg level in lake trout appear to be as important as the effects of between-lake variation in methyl mercury exposure. Results from monitoring only one species as a means to assess the general level of Hg contamination in fish (e.g., Hakanson et al. 1990) should therefore be interpreted with caution before they are applied to other fish species or to the general contamination status of a lake.

The failure of the model to explain bioaccumulation of both PCBs (Rasmussen et al. 1990) and Hg (this study) in littoral species suggests that lakes are heterogeneous environments with regard to processes leading to the bioaccumulation of contaminants in fish. Apart from food chain biomagnification, these processes could also include spatial and temporal variation in Hg bioavailability at the base of the food chain. There is no systematic way to classify complex littoral food webs, and it is therefore difficult to separate variation in biomagnification through food chains of variable length from variation in bioavailability at the base of the food chain.

Our study echoes Rasmussen et al.'s (1990) warning about the introduction of pelagic forage species such as *Mysis*, smelt, or coregonids as a source of fish food in lakes previously devoid of them. This practice, which has been favored in the past (e.g., *Mysis*: Martinez and Bergersen 1989), will likely result in increases in the concentration of Hg (this study) and PCBs (Rasmussen et al. 1990) in the salmonids situated at the top of the pelagic food chain.

Reports of increased Hg concentration toward the top of aquatic food chains have almost always dealt with comparisons of species that differ not only taxonomically (e.g., fish, invertebrates, zooplankton, and phytoplankton) but also in body size and physiology (e.g., Boudou et al. 1979; Philips et al. 1980; Ribeyre et al. 1980). The interpretation of this increase in Hg upward through the food chain as being the result of transfer via food uptake has been criticized by Huckabee et al. (1979) who suggested that factors such as differences in longevity (duration of exposure to Hg), growth rates, uptake, and depuration rates among species belonging to different trophic levels could well be as important as food chain transfer. Here, we have related between-lake variation in Hg concentration within a single species (lake trout) to the length of the pelagic food chain leading to this top predator. While the selection of a single species should minimize the effects of the confounding factors discussed by Huckabee et al. (1979), some of the objections raised against the interspecific comparisons of Hg concentrations could also apply to between-population studies such as the present one. For example, differences in age of the specimens, potentially related to demographic differences between Class 1 and 3 lake trout, could explain our results if Class 3 trout were generally found to be older than Class 1 fish. In addition, as pointed out by deFreitas (1974), faster growth rates resulting in a growth dilution and therefore lower Hg concentration can also be a confounding factor in interspecific studies. This raises the possibility of a growth rate artifact in our study if Class 1 trout generally grow faster than Class 3 trout, leading to higher growth dilution and lower Hg concentration in Class 1 populations. However, both the age and growth rate explanations appear to be ruled out in our study because piscivorous populations of lake trout (Classes 2 and 3) have been shown to grow faster than planktivorous (Class 1) populations (Martin 1966). When body size is statistically held constant, as was done in our final model (eq. 1), Class 3 fish are younger than Class 1 fish, since they grow faster and have therefore been exposed to Hg for less time. If there is any importance to factors such as age and growth dilution, these would help obscure rather than favor the trends observed in the present study. Consequently, the importance of biomagnification up through the food chain from Class 1 to Class 3 populations may well be more important than revealed by the differences in mean Hg concentrations across the three food chain classes. This could explain the difference between our estimate of the average biomagnification factor per trophic level (1.95) and the higher value of 3.33 obtained by Meili (1991) for the water/seston–zooplankton–roach/perch–pike food chain.

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Phosphorus–Chlorophyll Relationships under Contrasting Herbivory and Thermal Stratification: Predictions and Patterns

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Analyses of the relationships between total phosphorus (TP) and chlorophyll *a* (Chl) among a large number of temperate lake ecosystems having contrasting herbivore communities and thermal stratification reveal that systems lacking large *Daphnia* (SH systems) exhibit four times more Chl yields than systems having large *Daphnia* (LH systems) in oligotrophic as well as eutrophic and hypereutrophic systems. Mixed (MIX) systems exhibit greater Chl yields than stratified (STR) systems. Within each group of stratified and mixed systems, greater Chl yields to constant TP are observed in systems lacking large *Daphnia* (MIX-SH and STR-SH) than those having large *Daphnia* (MIX-LH and STR-LH). Consequently, a hierarchy of Chl yield patterns is produced; STR-LH and MIX-SH systems exhibit the lowest and highest Chl yields, respectively, at constant TP. These patterns may be a reflection of variable chemical (nutrients), biological (herbivory), and physical (thermal stratification) characteristics among aquatic ecosystems. Sigmoid patterns of TP–Chl relationships appear to be related to the transition from stratified to mixed systems along the TP gradient. The finding that highly variable Chl yields to TP can be summarized into a set of TP–Chl trajectories for specific types of lake ecosystems may have strong implications for lake management.

L'analyse des relations entre le phosphore total (TP) et la chlorophylle *a* (Chl) parmi un grand nombre d'écosystèmes de lacs tempérés possédant des communautés herbivores et une stratification thermique différentes révèle que les systèmes dont sont absentes les *Daphnia* de grande taille (systèmes SH) présentent des productions de Chl quatre fois plus fortes que les systèmes où se trouvent des *Daphnia* de grande taille (systèmes LH) dans des lacs aussi bien oligotrophes qu'eutrophes et hypereutrophes. Les systèmes mélangés (MIX) présentent des productions de Chl supérieures à celles des systèmes stratifiés (STR). Dans chaque groupe de systèmes stratifiés et mélangés, on observe des productions plus grandes de Chl par rapport au TP constant dans les systèmes dont sont absentes les *Daphnia* de grande taille (MIX-SH et STR-SH) que dans ceux où se trouvent des *Daphnia* de grande taille (MIX-LH et STR-LH). En conséquence, il s'établit une hiérarchie des profils de production de Chl, les systèmes STR-LH et MIX-SH présentant respectivement les productions les plus basses et les plus élevées de Chl, à un niveau constant de TP. Ces profils peuvent correspondre à des caractéristiques variables sur le plan chimique (nutriants), biologique (herbivores) et physique (stratification thermique) entre les écosystèmes aquatiques. Les profils sigmoïdes des relations TP–Chl semblent liés à la transition des systèmes stratifiés aux systèmes mixtes le long du gradient de TP. La découverte du fait que la très grande variabilité du rapport de la production de Chl au TP peut se résumer à une série de trajectoires TP–Chl pour des types spécifiques d'écosystèmes lacustres peut avoir des incidences importantes pour la gestion des lacs.

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Empirical relationships between total phosphorus (TP) and chlorophyll *a* (Chl) are used as evidence supporting the regulation of algal biomass by phosphorus (Sakamoto 1966; Vollenweider 1968; Dillon and Rigler 1974; Schindler et al. 1971). However, these relationships typically exhibit as much as two orders of magnitude of variation in Chl at a constant TP (Chl yield) (e.g., Smith 1982; McCauley et al. 1989; Prairie et al. 1989; Watson et al. 1992). To explain this high variance in Chl yields, many physical, chemical, and biological variables have been used (see review by Smith 1990).

An equally impressive set of literature documents grazer control of algal biomass. It has been suggested that herbivorous zooplankton can control algal biomass (Hrbáček et al. 1961; Brooks and Dodson 1965; Shapiro 1980) and that algal biomass may be regulated by strong interactions between nutrients and herbivory (McQueen et al. 1986; Mazumder et al. 1988, 1992b; Power 1992; Sarnelle 1992). However, the hypothesis that herbivory can significantly modify TP–Chl relationships has been tested by relatively few investigators (Shapiro 1980; Pace 1984; Hansson 1992; Sarnelle 1992), especially on a widest possible range of TP (Quirós 1990).

Lake morphometry (Straskraba 1978, 1980; Smith 1990) and the presence and absence of thermal stratification (Riley and Prepas 1985) have been suggested as important determinants of algal biomass. Deep stratified lakes are fundamentally different from shallow mixed lakes. Sedimenting algae and nutrients are lost to hypolimnia of stratified lakes, while they are often resuspended in mixed lakes. Reminer-

TABLE 1. General description of the TP and Chl data used in this study (both as $\mu g \cdot L^{-1}$).[a]

	Stratified having large *Daphnia* (STR-LH)			Stratified lacking large *Daphnia* (STR-SH)			Mixed having large *Daphnia* (MIX-LH)			Mixed lacking large *Daphnia* (MIX-SH)		
	TP	Chl	Chl:TP	TP	Chl	Chl:TP	TP	Chl	Chl:TP	TP	Chl	Chl:TP
n		81			148			50			88	
Minimum	4.9	0.2	0.04	1.0	0.5	0.50	17.9	2.8	0.16	23.7	6.3	0.27
Maximum	75.9	12.0	0.16	165.9	72.1	0.43	995.4	219.8	0.22	990.8	920.4	0.93
Mean	10.8	1.8	0.17	9.0	4.6	0.51	97.0	16.4	0.16	112.4	77.4	0.69

[a]Sources of data: (1) whole-lake experiments (Hrbáček et al. 1961; Shapiro 1979, 1980; Shapiro et al. 1982; Shapiro and Wright 1984; Hyatt and Stockner 1985; Stockner and Shortreed 1985; Hardy et al. 1986; Wagner 1986; Brabrand et al. 1987; Gulati 1990; Faafeng and Brabrand 1990; Lyche et al. 1990; Reinertsen et al. 1990; Sanni and Wærvågen 1990; Jeppesen et al. 1990a, 1990b; Søndergaard et al. 1990; van Donk et al. 1990a, 1990b, 1990c; van Liere et al. 1990; Boers et al. 1991; Carpenter et al. 1991); (2) enclosure experiments (Shapiro 1980; Drenner and Smith 1990; Mazumder et al. 1988, 1990b, 1992b); (3) comparative lake studies (Hyatt and Stockner 1985; Scholz et al. 1985; Stockner and Shortreed 1985; Hardy et al. 1986; Lyche et al. 1990; Mazumder et al. 1990a, 1990b, 1990c, 1992b; Richman and Sager 1990; Richman et al. 1990; Carpenter et al. 1991); (4) Lake Washington (18 yr of data) (Edmondson and Lehman 1981; Edmondson and Litt 1982); (5) Lake Ontario (1972 and 1982) (Lean et al. 1990; Mazumder et al. 1992a). A detailed data set can be obtained from the author upon request.

alization of nutrients differs significantly among stratified and mixed lakes (Levine and Schindler 1992). Large herbivorous zooplankton can escape from visual predators by seeking refuge in the hypolimnia of stratified lakes during daytime and can graze on the epilimnetic algae at nighttime (Wright and Shapiro 1990). Riley and Prepas (1985) found that mixed lakes in Alberta exhibit a greater Chl yield than stratified lakes; the range of TP exhibited by their data set was 8–156 $\mu g\ TP \cdot L^{-1}$. It is therefore important to examine if the patterns of TP–Chl relationships observed by Riley and Prepas exist for a wide range of TP. It is also important to examine if the influence of herbivores on TP–Chl relationships is more, or less pronounced in stratified systems compared with that in mixed systems.

The main purpose of the study is to evaluate the importance of contrasting herbivory indicated by the presence and absence of large *Daphnia* and of contrasting thermal stratification indicated by the presence and absence of permanent stratification during summer months as factors influencing TP–Chl relationships. More specifically, I examine whether the large variation in Chl yields is actually made up of a family distinguishable TP–Chl trajectories associated with the contrasts in herbivory and thermal stratification. My expectation is based on the observation that constant nutrient concentration produces low Chl yields when large grazers are present (Mazumder et al. 1988, 1992b). According to Oksanen et al. (1981) and McQueen et al. (1986), grazer effect on algal biomass is strongest in oligotrophic lakes and weakest in eutrophic and hypereutrophic lakes. Therefore, contrasting *Daphnia* communities should produce smaller differences in Chl yields among eutrophic and hypereutrophic systems. Given the differences in nutrient dynamics and loss processes between stratified and mixed systems, I also expect to observe higher Chl yields in mixed systems than in stratified systems (Straskraba 1980; Riley and Prepas 1985). As I use data from both natural and experimental ecosystems in my analyses, it is essential to test whether the TP–Chl relationship produced by experimental systems is significantly different from that produced by natural systems. A need for such a comparison comes from notion that results from experimentally manipulated systems are seldom comparable with results from natural systems because experimental ecosystems are "unstable" and "transient" (Crowder et al. 1988).

Materials and Methods

Data were collected from a large number of experimental and comparative studies of north temperate systems (lakes and enclosures) in north America and Europe (Table 1). Selection of literature data was based on the criteria that (1) samples were collected from the entire water column of mixed systems and from the epilimnion of stratified systems, (2) data included spring to summer means (May to August) based on weekly, biweekly, or monthly samples, (3) data indicated the size or community structure (density or biomass) of zooplankton communities or of herbivorous zooplankton, and (4) data indicated presence or absence of thermal stratification during summer. Lakes and enclosures (systems) were classified into two groups: (1) systems having large herbivores, i.e., *Daphnia* >1 mm mean length (LH systems) and (2) systems lacking large *Daphnia* (SH systems). *Daphnia* are considered large when their mean lengths exceed 1 mm (McQueen and Post 1988). The mean length of large *Daphnia* (e.g., *D. pulex*, *D. magna*, *D. longispina*, *D. galeata mendotae*, *D. hyalina*, *D. pulicaria*) ranges from 0.9 to 3.0 mm (Brooks 1957), and these species are seldom encountered when the biomass of predators (planktivorous fish) exceeds $20\ kg \cdot ha^{-1}$ (Mills and Schiavone 1982; Mills et al. 1987; McQueen and Post 1988; Jeppesen et al. 1990a; Søndergaard et al. 1990). Conversely, smaller species of *Daphnia* (e.g., *D. cuculatta*, *D. ambigua*, *D. longiremis*, *D. cristata*, *D. retrocurva*; see Brooks 1957) can coexist with high biomass of planktivorous fish (Brooks and Dodson 1965; Gulati 1990; Lyche et al. 1990; A. Mazumder, personal observation). Generally, zooplankton communities are represented by smaller cladocerans (*Bosmina*, 0.2–0.4 mm; *Ceriodaphnia*, 0.3–0.5 mm), rotifers (0.06–0.2 mm), cyclopoid copepods (0.4–1.0 mm), and nauplii (0.1–0.3 mm) in high-planktivore systems (Brooks and Dodson 1965; Mazumder et al. 1990a, 1992b), and these zooplankton are less efficient consumers of algae (Lehman and Sandgren 1985; Knoechel and Holtby 1986a, 1986b; Lampert et al. 1986; Mazumder et al. 1990a). Although the mean length

of *Holopedium*, a cladoceran, can exceed 1 mm, I did not consider the systems having *Holopedium* as LH systems because they can coexist with abundant planktivorous fish and high algal biomass (A. Mazumder and D.R.S. Lean, unpublished data).

An ecosystem was classified as SH system if (1) large *Daphnia* (*D. pulex*, *D. magna*, *D. longispina*, *D. galeata mendotae*, *D. hyalina*) were absent or low in numbers (<5 *Daphnia*·L^{-1} or <20% of total zooplankton biomass or <10% of crustacean abundance) (Brooks and Dodson 1965; Reinertsen et al. 1990), (2) cladoceran mean length was <0.5 mm (Gulati 1990; Mazumder et al. 1990, 1990a, 1990c), (3) *Daphnia* mean length was <1.0 mm (McQueen and Post 1988; Mazumder et al. 1990b, 1990c), and (4) biomass of planktivorous fish was >20 kg·ha^{-1} (Mills and Schiavone 1982; Mills et al. 1987; McQueen and Post 1988). The rationale for using cladoceran mean length <0.5 mm as small herbivores (criterion 2) is that the mean length of small cladocerans such as *Bosmina* and *Ceriodaphnia* ranges from 0.2 to 0.5 mm, and therefore, other cladocerans such as large *Daphnia* have to be dominant to produce a mean length >0.5 mm (Mazumder et al. 1990b, 1990c). The last criterion was based on the minimum biomass of planktivorous fish above which large herbivores (especially large *Daphnia* are rarely encountered, although critical levels ranging from 20–30 kg·ha^{-1} (Mills et al. 1987), 30–50 kg·ha^{-1} (McQueen and Post 1988), and >150 kg·ha^{-1} (Søndergaard et al. 1990) to >600 kg·ha^{-1} (Jeppesen et al. 1990a) have been suggested. Nevertheless, data on planktivorous fish were used only as a supplementary information, while my classification was mainly based on the presence and absence of large *Daphnia*. Based on these criteria, 236 data points were considered as SH systems as indicated by an absence or a near absence of large *Daphnia*. Each of these 236 data points showed at least one of the following characteristics: (1) *Daphnia* were absent, (2) *D. cuculatta* (adult size 0.4–0.7 mm) were present, (3) *D. ambigua* or *purvula* were present, but <0.7 mm, (4) *D. retrocurva* were present, but <0.8 mm or <2% of *D. retrocurva* were >1.0 mm, (5) rotifers and copepods made up >90% of total zooplankton biomass, (6) cladoceran mean lengths were <0.5 mm, and (7) *Daphnia* (mostly *D. cuculatta*) made up <20% of zooplankton biomass. For 215 out of 236 data points, reported or calculated (from abundance) biomass of planktivorous fish was >20 kg·ha^{-1}.

An ecosystem was classified as LH system if (1) at least one species of large *Daphnia* (*D. pulex*, *D. magna*, *D. longispina*, *D. galeata mendotae*, *D. hyalina*, *D. pulicaria*) was present (>5 *Daphnia*·L^{-1} or >20% of zooplankton biomass or >10% of crustacean abundance), (2) *Daphnia* mean length was >1.0 mm, (3) cladoceran mean length was >0.5 mm, and (4) planktivorous fish biomass was <20 kg·ha^{-1}. Based on these criteria, 131 ecosystems were classified as LH systems. Each of these 131 data points showed at least one of the following characteristics: (1) the abundance of large *Daphnia* (at least one of the six species above) ranged from 8 to 500·L^{-1}, (2) large *Daphnia* contributed 40–90% to zooplankton biomass, (3) *Daphnia* mean length ranged from 1.0 to 2.6 mm, (4) the cladoceran mean length ranged from 0.52 to 0.92 mm, and (5) planktivorous fish were absent or biomass was <20 kg·ha^{-1}.

The criteria I used to categorize systems having large *Daphnia* from those lacking large *Daphnia* could be improved for future studies. Data indicating herbivore community structures were not consistent in all studies used here; some indicated biomass, others indicated abundance, while others still presented data on *Daphnia* only. As a result, I could not use abundance or biomass of herbivores or of *Daphnia* as a continuous variable. Nevertheless, I have been consistent in my classification of systems by the presence and absence of large *Daphnia*.

Altogether I have analyzed 367 lake-years of data from ultraoligotrophic to hypereutrophic systems with contrasting herbivorous zooplankton communities and thermal stratification. Out of 367 lake-years of data, 15 were from experimental enclosures, 198 were from whole-lake manipulations, and 154 were from natural (unmanipulated) lakes. One year (spring through summer mean) of data from a system have been used as one lake-year and represent a data pair for TP and Chl. All the data were categorized into four system types (Table 1): (1) stratified having large herbivores (STR-LH), (2) stratified lacking large herbivores (STR-SH), (3) mixed having large herbivores (MIX-LH), and (4) mixed lacking large herbivores (MIX-SH).

Statistical Analyses

Statistical analyses of the data were done using Systat, version 5.1 (Wilkinson 1991). As most of the published models on TP–Chl relationships have been based on linear models (ordinary least squares regression) of $\log_{10}$-transformed or $\log_{10}$-scaled data, I have used the same method to compare equations between contrasting system types, for example, SH versus LH, STR versus MIX, STR-SH versus STR-LH, and MIX-SH versus MIX-LH systems. The differences among linear models were tested by comparing slopes and intercepts. In order to avoid the problem of prejudging the nonlinearity of the relationship between TP and Chl by fitting a second- or third-order polynomial model, I used the locally weighted sequential smoothing (LOWESS algorithm) to determine the patterns of TP–Chl relationships. This type of scatter plot smoothing allows one to look for a functional relationship between Y and X without prejudging its shape. The method for finding smoothed values involves a locally weighted robust regression, where regressions are performed within a small range of independent variable and all the individual linear fits are connected to form the shape of the relationship for the entire range. A similar method has been used by McCauley et al. (1989) and Prairie et al. (1989). To estimate the effects of *Daphnia* and stratification on Chl, ANCOVA was performed with two levels for *Daphnia* (presence versus absence), two levels for stratification (stratified versus mixed), and TP as a covariate. Multivariate regression analyses were performed to evaluate the importance of additional independent variables such as total nitrogen (TN), herbivory, and stratification in explaining the variance remaining unexplained by the TP–Chl model.

Results and Discussion

As expected, the concentrations of Chl and TP produce a highly significant positive relationship (Fig. 1a). The least squares regression equation for the whole data set is

$$(1) \qquad \log \text{Chl} = -0.35 \pm 0.05 + (0.94 \pm 0.03) \log \text{TP};$$
$$n = 367,\ r^2 = 0.71.$$

The slope of the equation is 1 ($P < 0.0001$) and the y-intercept is < 0 ($P < 0.0001$). Errors are 1 SE of the slope and y-intercept. The slope and y-intercept are similar to other published relationships based on a similar range of TP (Prairie et al. 1989).

Statistically, the TP–Chl relationship derived here (equation (1)) is highly satisfactory because it explains a significant amount (71%) of variance in Chl yields. Ecologically too, it is a meaningful relationship, suggesting that TP is a robust indicator of Chl yields among a large number of widely variable north temperate systems. However, the yields of Chl at a given TP vary as much as two orders of magnitude (Fig. 1a); this magnitude of variation is the same as that observed by others (McCauley et al. 1989; Prairie et al. 1989; Watson et al. 1992). Consequently, for the practical purpose of lake management, if one wishes to predict the Chl yield in a specific lake, the predicted range derived from equation (1) may be misleading. For example, equation (1) suggests that a lake with a TP of 50 μg TP·L^{-1}, a value exhibited by all four system types in my data set, may produce Chl yields ranging from 14 to 22 μg Chl·L^{-1} (predicted mean ± SE), a less than twofold variation; however, the actual data set indicates 3–67 μg Chl·L^{-1}, a 22-fold variation (Fig. 1a). I will use the example of 50 μg TP·L^{-1} repeatedly to try to predict the Chl yield in a specific lake with more reliability using the additional characteristics such as the contrasts in large *Daphnia* and thermal stratification.

Traditionally, we try to explain more of the unexplained variance in Chl yields by adding additional independent variables to TP–Chl relationships (Smith 1982; Pace 1984; Riley and Prepas 1985; McCauley et al. 1989; Quirós 1990). Below, I examine whether there is a specific structure in the distribution of the data points on the TP–Chl scatter plot (Fig. 1a) and whether certain types of systems (based on the contrasts in large *Daphnia* and thermal stratification) produce extreme outliers. If the latter can be demonstrated, then a general model such as equation (1) or a multivariate model explaining a greater percentage of the variance in Chl yields may selectively exclude the specific types of systems producing extreme outliers. My approach is quite logical considering the potentially variable nature of phosphorus dynamics in aquatic ecosystems of widely variable morphometry (Straskraba 1980; Levine and Schindler 1992) and consumer communities (Mazumder et al. 1992b).

TP–Chl Relationships under Contrasting Herbivory

The clearest pattern revealed by the TP–Chl scatter plot is the distinct separation of systems lacking large *Daphnia* (SH) from the systems having large *Daphnia* (LH) (Fig. 1b): SH systems are scattered above the LH systems with very little overlapping. The significant difference of Chl yields in SH versus LH systems is indicated by the results of ANCOVA testing the effect of the presence and absence of large *Daphnia* with TP as a covariate (Table 2). Ordinary least squares regressions produce models that support a distinct separation of the two types of systems with contrasting large *Daphnia* communities. Systems lacking large *Daphnia* (SH) yield

$$\log \text{Chl} = -0.21 \pm 0.03 + (0.97 \pm 0.02) \log \text{TP}; \quad (2)$$

$$n = 236,\ \text{TP} = 1\text{–}991\ \mu\text{g}\cdot\text{L}^{-1},\ r^2 = 0.87.$$

In systems having large *Daphnia* (LH), the equation is

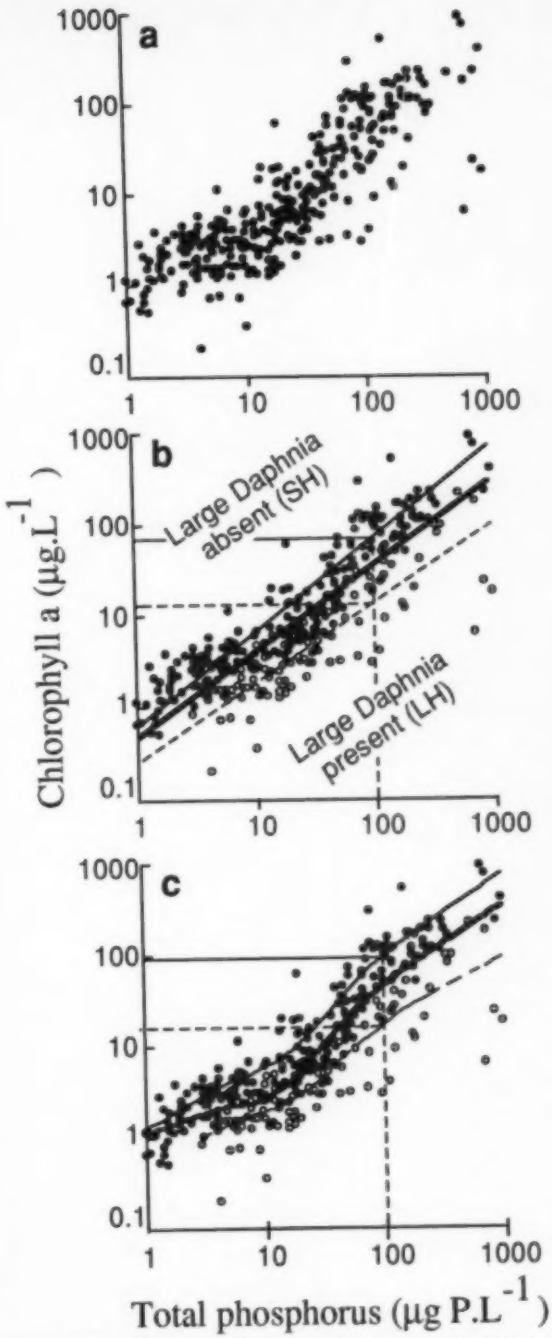

FIG. 1. Relationships between TP and Chl for (a) the whole data set used in this study (Table 1) and (b) two types of systems: SH systems lacking large *Daphnia* (solid symbols) and LH systems having large *Daphnia* (open symbols). The thick solid line is the least squares regression fit for the whole data set (equation (1) in the text) and the thin solid line is for SH systems (equation (2) in the text). The thin broken line is the same, but for LH systems (equation (3) in the text). (c) Same as in Fig. 1b but showing the LOWESS patterns fitted through SH and LH systems separately and both groups together.

TABLE 2. ANCOVA indicating the effects of large *Daphnia* (two levels, presence versus absence) and stratification (two levels, presence versus absence) on $\log_{10}$ Chl with $\log_{10}$ TP as a covariate. r^2 and R^2 are the coefficients of determination for univariate (TP) and multivariate (herbivores and/or stratification with TP as a covariate) models, respectively. Number of observation is constant for all three models (n = 367).

Model	df	F (partial)	P (partial)	r^2	R^2
(a) Influence of thermal stratification					
Statification	1	34.3	<0.0001	0.71	0.75
$\log_{10}$TP (covariate)	1	270.9	<0.0001		
Error	364				
(b) Influence of large *Daphnia*					
Daphnia	1	274.6	<0.0001	0.71	0.85
$\log_{10}$TP (covariate)	1	1707.2	<0.0001		
Error	364				
(c) Influence *Daphnia* and stratification					
Daphnia	1	308.6	<0.0001	0.71	0.87
Stratification	1	44.6	<0.0001		
Daphnia·stratification	1	7.1	0.0081		
$\log_{10}$TP (covariante)	1	535.2	<0.0001		
Error	362				

(3) $\log \text{Chl} = -0.60 \pm 0.07 + (0.86 \pm 0.05) \log \text{TP}$;
$n = 131$, TP = 5–995 $\mu g \cdot L^{-1}$, $r^2 = 0.73$.

SH systems produce a slightly steeper slope but a significantly higher y-intercept than LH systems, indicating that the former produce greater Chl yields per unit TP than the latter. In Fig. 1b, the linear fit for SH is on average 0.4 log unit above that for LH systems. Others (Shapiro 1979, 1980; Pace 1984; Quirós 1990; Hansson 1992; Sarnelle 1992) have also observed greater Chl yields per unit TP among lakes lacking large *Daphnia*. Equations (2) and (3), based on the presence and absence of large *Daphnia*, significantly improve our ability to predict Chl yields at any specific concentration of TP. Reconsidering the example of 50 μg TP·L^{-1}, if this specific lake lacks large *Daphnia*, equation (2) predicts Chl yields from 14 to 23 μg Chl·L^{-1}, and if it has large *Daphnia*, equation (3) predicts Chl yields from 5.9 to 10.4 μg Chl·L^{-1} (Fig. 2b). Consequently, it is now possible to predict over a fourfold variation in Chl yields (5.9–23 μg Chl·L^{-1}) at a constant TP of 50 $\mu g \cdot L^{-1}$ whereas based on TP alone (equation (1)), I could explain less than twofold variation in Chl yields (14–22 μg Chl·L^{-1}). Although these two equations for SH and LH systems improve predictive power, the fourfold predicted range is still only a small part of the total variation.

More importantly, however, the two approximately parallel linear fits indicate that a greater Chl yield per unit TP in SH systems (equation (2)) than in LH systems (equation (3)) is consistent in oligotrophic as well as eutrophic and hypereutrophic systems (Fig. 1b). Oksanen et al. (1981) and McQueen et al. (1986) hypothesized that herbivore effect on Chl concentration is strongest among oligotrophic lakes and weakest among eutrophic and hypereutrophic lakes; this hypothesis would predict that the linear fits for SH and LH systems should converge at high TP concentration. The linear fits for SH and LH systems do not converge (Fig. 1b; equations (2) and (3); on the contrary, they tend to diverge, although not significantly. The ranges of TP exhibited are not very different for SH (1–991 μg TP·L^{-1}) and LH (5–995 μg TP·L^{-1}) (Table 1) and therefore, the slopes and y-intercepts produced by these two types of systems may not be influenced by the ranges of TP. In addition, results of ANCOVA (Table 2) and LOWESS (Fig. 1c; discussed below) appear to support my interpretation that significantly lower Chl yields in the LH systems than in SH systems are observed in oligotrophic as well as hypereutrophic systems.

Although I have used linear regressions to compare TP–Chl relationships, the actual patterns of the relationships seem to be sigmoidal (Fig. 1c), as also observed recently by others (McCauley et al. 1989; Prairie et al. 1989; Watson et al. 1992). The patterns produced by LOWESS analysis also suggest that the higher Chl yields in SH systems than in LH systems are proportionately similar regardless of the trophic status. The potential mechanisms associated with sigmoid patterns of TP–Chl relationships are discussed later.

TP–Chl Relationships under Contrasting Thermal Stratification

Results of ANCOVA with stratification (presence versus absence) as a factor and TP as a covariate indicated significantly different (higher) Chl yields per unit TP in mixed systems than in stratified systems (Table 2). Comparison of the TP–Chl relationships between stratified and mixed systems is complicated by the significantly different scale of TP exhibited by the two types of systems (Table 1). In this data set, TP ranged from 1 to 165.9 $\mu g \cdot L^{-1}$ among the stratified systems and from 18 to 995 $\mu g \cdot L^{-1}$ among the mixed systems. On average, mixed systems have an order of magnitude higher mean TP than the stratified systems. The problem of scale effect was minimized using three approaches. Firstly, the least squares regressions were performed within same range of TP for the two types of systems. Secondly, Chl was predicted as a function of TP (assuming zero intercept), and slopes were compared statistically. Thirdly, the patterns of TP–Chl relationships for stratified and mixed systems are derived using LOWESS which are not influenced by the range of the independent variable. Prior to adjustment of TP range, the least squares regression for stratified system is

(4) $\log \text{Chl} = -0.15 \pm 0.05 + (0.68 \pm 0.05) \log \text{TP}$;
$n = 229$, $r^2 = 0.87$

and for mixed systems is

(5) $\log \text{Chl} = -0.14 \pm 0.20 + (0.88 \pm 0.09) \log \text{TP}$; $n = 138$, $r^2 = 0.39$.

For a TP range of 18–165 $\mu g \cdot L^{-1}$, stratified systems produce the equation

(4a) $\log \text{Chl} = -0.70 \pm 0.20 + (1.09 \pm 0.14) \log \text{TP}$; $n = 92$, $r^2 = 0.41$

and mixed systems the equation

(5a) $\log \text{Chl} = -1.08 \pm 0.31 + (1.41 \pm 0.16) \log \text{TP}$; $n = 91$, $r^2 = 0.45$.

Following adjustment for TP range, least squares regression equations for both the stratified and mixed systems change substantially. A similar effect of scale on the slope and *y*-intercept of the TP–Chl relationship has been observed by Riley and Prepas (1985) for Alberta lakes. However, equations derived from both unadjusted and adjusted ranges of TP indicate that the rate of increase in Chl yield per unit increase of TP is greater (larger slope) in mixed systems than in stratified systems. The equations, log Chl = *b*(log TP) assuming zero intercept, also produce significantly larger slope for mixed systems (0.82 ± 0.02) than that for stratified systems (0.55 ± 0.02). On average, mixed systems have 1.5 times more Chl yield per unit TP than the stratified systems (Table 1). The larger slope of the TP–Chl relationship for mixed systems indicates a greater Chl yield per unit increase in TP when the water columns of lakes are not stratified compared with when the water columns are stratified. This is consistent with the observations of Riley and Prepas (1985).

Patterns of TP–Chl Relationships under Contrasting Herbivory and Stratification

Analysis of TP–Chl relationships under contrasting communities of large *Daphnia* within each group of stratified and mixed systems produces a hierarchy of Chl yields per unit TP (Fig. 2a). These hierarchical patterns of TP–Chl relationships are supported by both least squares regression fits and LOWESS trends. Stratified systems lacking large *Daphnia* (STR-SH) produce

(6) $\log \text{Chl} = -0.01 \pm 0.06 + (0.69 \pm 0.05) \log \text{TP}$; $n = 118$, $r^2 = 0.57$, TP = 1–76

and those having large *Daphnia* (STR-LH) yield

(7) $\log \text{Chl} = -0.44 \pm 0.10 + (0.67 \pm 0.10) \log \text{TP}$; $n = 81$, $r^2 = 0.36$, TP = 4.9–76.

Among stratified systems, both STR-SH and STR-LH systems have similar slopes, but the linear fit for the former is 0.4 log unit higher than STR-LH systems. The *y*-intercept for STR-SH systems is not different from zero ($P = 0.909$), while it is smaller than zero for STR-LH systems ($P < 0.0001$). The slopes of both the equations are smaller than 1 ($P < 0.0001$), but not different from each other. This is consistent with a three times greater Chl yield per unit TP in STR-SH systems than in STR-LH systems (Table 1). ANCOVA for the effects of herbivore (STR-SH versus STR-LH) as a factor and TP as a covariate is also highly significant ($P < 0.0001$) (Table 2).

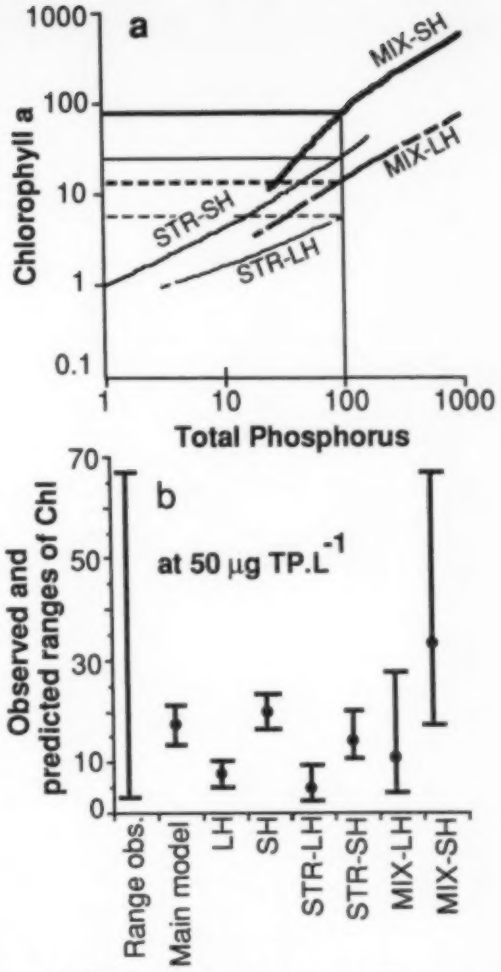

FIG. 2. (a) LOWESS trends produced by stratified systems having large *Daphnia* (STR-LH), stratified systems lacking large *Daphnia* (STR-SH), mixed systems having large *Daphnia* (MIX-LH), and mixed systems lacking large *Daphnia* (MIX-SH). These four LOWESS trends illustrate the hierarchy of the Chl yield per unit TP and correspond to linear equations (6)–(9). (b) Ranges of Chl yields observed and predicted (mean ± 1 SE) at a constant TP of 50 $\mu g \cdot L^{-1}$ by using the linear equations for the whole data set (main model, equation (1)) and separately for each of groups of systems. Abbreviations on the *x*-axis correspond to system types described in Fig. 1b and 2a (equations (2), (3), and (6)–(9)).

Mixed systems lacking large *Daphnia* (MIX-SH) produce an equation of

(8) $\log \text{Chl} = -0.28 \pm 0.17 + (1.06 \pm 0.08) \log \text{TP}$; $n = 88$, $r^2 = 0.67$, TP = 24–991

and those having large *Daphnia* produce

(9) $\log \text{Chl} = -0.06 \pm 0.22 + (0.64 \pm 0.11) \log \text{TP}$; $n = 50$, $r^2 = 0.43$, TP = 18–995.

Unlike the stratified systems, MIX-SH systems produce a significantly larger slope (slope = 1) than MIX-LH systems (slope < 1). The *y*-intercepts of both equations are not dif-

ferent from zero. Among these four types of systems, the increase in Chl yield per unit increase in TP is most rapid (largest slope) in the MIX-SH systems where Chl and TP tend to increase following a 1:1 ratio.

A hierarchical elevation of TP–Chl relationships among four types of systems is nicely illustrated by fitting LOWESS trends through the data points for each group of systems separately (Fig. 2a). In Fig. 2a, note that at a constant TP (at 100 μg $TP \cdot L^{-1}$), STR-LH systems exhibit the lowest Chl yields (<10 μg $Chl \cdot L^{-1}$) followed by the MIX-LH and STR-SH systems, and the MIX-SH systems exhibit the highest Chl yields (~80 μg $Chl \cdot L^{-1}$). These hierarchical patterns may be a reflection of the fundamentally different algal responses to constant TP associated with the variable nature of processes such as sedimentation, grazing, and remineralization of phosphorus among lake ecosystems with contrasting large *Daphnia* communities and thermal stratification. Based on the observed TP–Chl trajectories of four types of systems, it may be suggested that the same concentration of TP produces the highest Chl yields when the water column remains mixed and large *Daphnia* are lacking and the lowest Chl yields when the water column is stratified and large *Daphnia* are present. The hierarchical patterns produced by the four types of systems (Fig. 2) also indicate that the MIX-SH and STR-LH systems tend to produce the extreme outliers (highs and lows, respectively) among the systems analyzed in this paper (Fig. 1a).

For the hypothetical lake containing 50 μg $TP \cdot L^{-1}$, the four least squares regression equations (equations (6)–(9)) predict Chl yields ranging 2.7–9.3, 10.7–20.3, 4.2–27.2, and 20.6–67.1 μg $\cdot L^{-1}$ in STR-LH, STR-SH, MIX-LH, and MIX-SH systems, respectively (Fig. 2b). Therefore, the ranges of Chl yields predicted by the four submodels cover the entire range of observed yields (3–67 μg $Chl \cdot L^{-1}$) at 50 μg $TP \cdot L^{-1}$. Thus, if one knows whether a specific lake is stratified or mixed and whether it has large *Daphnia* or not, it is now possible to predict a more precise range of Chl yields than heretofore possible.

Significance of the different Chl yields in systems with contrasting large *Daphnia* and thermal stratification is also indicated by the results of ANCOVA with TP as a covariate (Table 2). The results of ANCOVA indicate that there is a significant interaction of herbivory and stratification effects on Chl yields. TP, contrasts in large *Daphnia*, and thermal stratification explain 87% of the variance in Chl yields. I realize that explaining 16% more variance by adding herbivory and stratification as independent variables to the TP–Chl relationship may mean the same as developing the submodels for each group of functionally different systems. However, by identifying the data points on the TP–Chl scatter plot (Figs. 1b and 2a) and by developing system-specific submodels, it has been possible to reveal the structure of this data set. As the MIX-SH and STR-LH systems tend to produce the extreme outliers, it is possible that 13% unexplained variance in Chl yields may be represented largely by these two types of systems. By developing TP–Chl trajectories for each group of functionally different ecosystems, it has been possible to predict Chl yields for individual lake types which will include the extreme outliers in a data set (Fig. 2b). Discovery of these vertically distinguishable trajectories of Chl yields may have important implications for lake management where lake managers would like to know the Chl yield in a specific lake.

TABLE 3. Univariate and multivariate least squares regressions indicating the relationships of $\log_{10}$ TP and/or $\log_{10}$ TN with $\log_{10}$Chl yields using a limited data set (n = 143).

Variable	Coefficient	SE	P (two-tailed)	r^2
Constant	−0.149	0.074	0.047	0.55
log TP	0.699	0.053	0.000	
Constant	−2.783	0.235	0.000	0.61
log TN	1.383	0.092	0.000	
Constant	−2.011	0.273	0.000	0.67
log TP	0.345	0.072	0.000	
log TN	0.924	0.132	0.000	

Comparison of TP–Chl Models in Natural and Experimental Systems

As I have used data from both natural and experimental systems to examine the patterns of TP–Chl relationships, it is important to test whether the observed patterns are an artefact of such a combination of systems. This test is particularly important because it has been suggested for example that manipulated ecosystems are unstable or transient because of the extreme manipulations of either nutrient loading or predator communities (Crowder et al. 1988). Out of 367 observations used in my analysis, 213 are from experimental systems where nutrient loading and/or planktivorous fish have been manipulated, and 154 observations are from natural or unperturbed systems. The ranges of TP are 1–350 and 1–977 μg $TP \cdot L^{-1}$ for natural and experimental systems, respectively. To make a comparison of TP–Chl models among natural versus experimental systems, but within a similar range of TP, the data with TP > 350 μg $TP \cdot L^{-1}$ are excluded from this analysis. Least squares regression produces an equation of log Chl = $-0.17 \pm 0.06 + 0.90 \pm 0.04$(log TP) for experimental systems (n = 198, r^2 = 0.76) which is similar to the equation for natural systems (log Chl = $-0.37 \pm 0.07 + 0.97 \pm 0.06$ (log TP); n = 154, r^2 = 0.65). For both equations, slopes are not different from 1 (P <0.005) and the intercepts are significantly different from zero (P <0.05). These two equations also have slopes and intercepts similar to estimations reported by others for natural systems (Pace 1984; Prairie et al. 1989). In addition, the LOWESS trend of the TP–Chl relationship, produced by McCauley et al. (1989) using natural ecosystems, corresponds nicely with that produced by the whole data set used in this study (Fig. 1c; LOWESS from McCauley et al. 1989, not shown). Similarly, Carpenter et al. (1991) compared TP, Chl, primary production, and zooplankton biomass among natural versus manipulated lakes and found that manipulated lakes were not distinctly more variable from year to year than natural lakes. More recently, Sarnelle (1992) has also found that data from experimental enclosures or lakes and natural lakes conform to the same TP–Chl relationship. My comparison of TP–Chl models for natural versus experimental systems suggests that data from experimental systems, although they are assumed to exhibit transient dynamics due to perturbation (Crowder et al. 1988), *do not* produce a TP–Chl model different from that based on data from natural systems; hence, I suggest that experimental systems produce results comparable with natural systems and that they complement in our search for understanding of aquatic ecosystems.

TABLE 4. ANCOVA indicating the influences of large *Daphnia* (two levels, presence versus absence) and stratification (two levels, presence versus absence) on $\log_{10}$ Chl yields with $\log_{10}$ TP and $\log_{10}$ TN as covariates. r^2 and R^2 are the coefficients of determination for univariate (TP) and multivariate (herbivores and/or stratification with TP as a covariate) models, respectively. Number of observations is 143.

Model	df	*F* (partial)	*P* (partial)	r^2	R^2
(c) Influence of *Daphian* and stratification					
Daphnia	1	103.8	<0.0001	0.55	0.84
Stratification	1	25.1	<0.0001		
Daphnia-stratification	1	6.8	0.0500		
TP (covariate 1)	1	68.7	<0.0001		
TN (covariate 2)	1	15.3	<0.0005		
Error	137				

Influence of Nitrogen as Second Nutrient

An impressive set of literature has documented total nitrogen (TN) as an important variable explaining a significant amount of variance in Chl yields (Smith 1979, 1982; McCauley et al. 1989; Prairie et al. 1989; Molot and Dillon 1991). Therefore, it is also essential to test whether the effects of TN are distinct and separable from the effects of herbivory and stratification found above. Out of 367 lake-years of data, 143 have data on TN, TP, herbivore community structure, and thermal stratification. The range of TP in this limited data set is the same as that of the whole data set. Individually, TP and TN explain 55 and 61% of the variance in Chl yields, respectively, suggesting that both nutrients are equally good predictors of Chl yields (Table 3). Addition of TN as a second independent variable to TP explains an additional 7% of the variance in Chl yields. Within this limited data set, contrasting large *Daphnia* as a second independent variable explains 17% more variance in Chl yields than TP alone ($r^2 = 0.72$). Stratification as a second independent variable explains 12% more variance ($r^2 = 0.67$). Both herbivory and stratification explain 27% more variance in Chl ($r^2 = 0.82$). Addition of TN along with herbivory and stratification as independent variables to TP–Chl model produces a coefficient of determination of 0.84 (Table 4). These analyses suggest that the Chl yields are determined by strong interactions of chemical (TP and TN), biological (herbivory), and physical (lake morphometry or thermal stratification) factors. However, the presence and absence of large *Daphnia* and thermal stratification explain more of the unexplained variance in TP–Chl relationship than does TN.

Sigmoidal Patterns of TP–Chl Relationships

The LOWESS analysis of TP–Chl relationships for stratified and mixed systems separately produces very interesting patterns (Fig. 3) which may be closely related to the sigmoidal pattern observed for the whole data set and also separately for SH and LH systems (Fig. 1c) and by others (McCauley et al. 1989; Prairie et al. 1989; Watson et al. 1992). The LOWESS pattern produced by the stratified systems is basically inverse to that by the mixed systems. The LOWESS fit for stratified systems begins with a flat TP–Chl relationship up to 10 μg TP·L^{-1} beyond which Chl yields increase rapidly with increasing TP. Conversely, the LOWESS fit for the mixed systems begins with a rapid increase in Chl yields up to 100 μg TP·L^{-1} beyond which Chl yields are smaller per unit increase in TP. Although the other studies

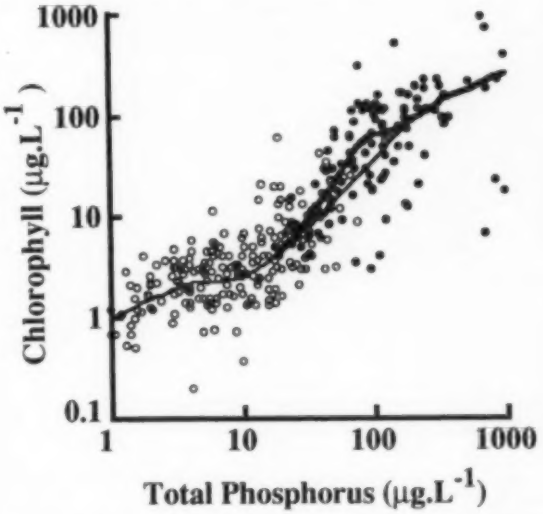

FIG. 3. TP–Chl relationships among stratified (open symbols) and mixed (solid symbols) systems (corresponding least squares regression equations (4)–(5b)). LOWESS trends for stratified (thin line) and mixed (thick line) systems are presented to illustrate the relationship between the sigmoidal pattern of the TP–Chl relationship produced by the whole data set (Fig. 1c) and the transition of system type from stratified to mixed systems along the TP gradient.

have yielded sigmoidal patterns in TP–Chl relationships, they have not looked into the nature of thermal stratification represented in their data sets (McCauley et al. 1989; Prairie et al. 1989; Watson et al. 1992).

It has been suggested that a sigmoidal relationship between TP and Chl may be due to the disproportionality of growth and loss rates of algae (McCauley et al. 1989) or to changes in the TN:TP ratio (Prairie et al. 1989) along the TP gradient of lakes. More recently, Watson et al. (1992) have suggested that a sigmoidal pattern is produced by a disproportional contribution of inedible algae to total algal biomass along the TP gradient. With the data set used here, I could not test the hypothesis of Watson et al. (1992). In a subsequent paper, I evaluate the role of grazers and nutrients in determining the size distribution of algae and associated TP–Chl relationships (Mazumder 1994). However, the data set analyzed here indicates that a sigmoidal pattern of TP–Chl relationships may also be due to the variable patterns of

Chl yields to TP in stratified versus mixed systems. My suggestion is well supported by the LOWESS trends for stratified and mixed systems separately (Fig. 3). Straskraba (1980) has suggested that a nonlinear TP–Chl relationship may be related to changes in mean depth of lakes along the TP gradient.

General Conclusions

This study made an attempt to explain the distribution patterns of Chl yields in north temperate systems by considering the importance of nutrient-, consumer- and physics-based models in a single study. My results not only show that TP and TN are the strongest correlates of Chl yield, but also that herbivores and thermal stratification can explain a significant amount of unexplained variance in Chl yields. More importantly, it has been possible to identify a set of vertically distinct TP–Chl trajectories indicating hierarchical Chl yield patterns to TP, and these patterns seem to be associated with the contrasts in large *Daphnia* and thermal stratification. Identifying the distinctly separable Chl yield patterns is as important as explaining more of the unexplained variance in Chl yields by using these two factors as additional independent variables because it identifies the types of ecosystems that tend to produce extreme outliers.

The patterns reported in this paper might be a useful starting point to build upon empirical models that could also provide processes associated with observed patterns. It might be concluded that Chl yields in north temperate lake ecosystems are determined by the interactions of chemical (nutrients), biological (grazers), and physical (thermal stratification) factors. However, more research will be necessary to identify the processes associated with the hierarchy of Chl yields observed in this study.

The approach I have used here could be improved substantially. For example, I have used herbivory (presence versus absence of large *Daphnia*) and thermal stratification (stratified versus mixed) as discrete categories to evaluate the importance of these two factors in influencing patterns of TP–Chl relationships, while the real world is continuous. Within each group of stratified or mixed systems having large *Daphnia*, there might be a large variation in the abundance and biomass of large *Daphnia* that I could not test because of the inconsistency of the data set. In addition, within stratified and mixed lakes, there must be a large variation in mean depth. It is possible that increasing Chl yield per unit TP is also related to decreasing mean depth along the TP gradient of lakes (Sakamoto 1966; Straskraba 1978, 1980; Fee 1979; reviewed in Smith 1990). I am presently analyzing a data set to use herbivory, stratification, and morphometry as continuous variables.

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Phosphorus–Chlorophyll Relationships under Contrasting Zooplankton Community Structure: Potential Mechanisms

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Data from experimental enclosures and natural and manipulated lakes were used to test whether the variable chlorophyll *a* (Chl) yields to total phosphorus (TP) can be explained by the variable contributions of dissolved (TDP) and zooplankton phosphorus (ZP; P > 200 μm) to TP. Results indicate that low Chl yields to TP in systems with abundant large *Daphnia* are closely related to greater contributions of TDP and ZP to TP. An opposite pattern is produced by systems lacking large *Daphnia*. These patterns seem to be consistent among mesoeutrophic to hypereutrophic systems; Chl yields to TP decline with increasing contributions of TDP to TP. In the large-*Daphnia*-dominated systems, both high grazing and reduced phosphate demand allow a greater sequestering of P into zooplankton and dissolved pools rather than in algae, which generate a lower observed Chl yields to TP. Conversely, lack of large *Daphnia* and low grazing rates allow proliferation of small algae, and an associated intense phosphate demand allows greater sequestering of P into small algae rather than into zooplankton and dissolved pools, and consequently, a higher Chl yield to TP is produced.

Des données provenant d'enceintes expérimentales, et de lacs naturels et traités ont été utilisées pour vérifier si les rapports de production variables de chlorophylle *a* (Chl) par rapport au phosphore total (TP) peuvent être expliqués par les proportions variables de phosphore dissous (TDP) et de phosphore zooplanctonique (ZP; P > 200 μm) composant le TP. Les résultats montrent que la faible production de Chl par rapport au TP de systèmes dans lesquels abondent des *Daphnia* de grande taille est étroitement liée à des proportions plus importantes de TDP et de ZP par rapport au TP. Un profil opposé est produit par des systèmes ne comportant pas de *Daphnia* de grande taille. Ces profils semblent être constants dans les systèmes mésoeutrophes et hypereutrophes; la production de Chl par rapport au TP diminue lorsque la part de TDP dans le TP augmente. Dans les systèmes où dominent des *Daphnia* de grande taille, le broutage élevé et la demande réduite en phosphates permettent un plus grand piégeage du P dans le zooplancton et les réserves de P dissous que dans les algues, ce qui entraîne une production plus faible de Chl par rapport au TP. Inversement, l'absence de *Daphnia* de grande taille et le faible taux de broutage permettent la prolifération de petites algues, et une forte demande en phosphates, un piégeage plus important de P dans de petites algues plutôt que dans le zooplancton et les réserves de P dissous, et par conséquent, une plus grande production de Chl par rapport au TP.

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Levin (1992) stated that "Understanding patterns in terms of processes that produce them is the essence of science and is the key to the development of principles for management". Power (1992) remarked that simple empirical predictive models may miss crucial mechanistic details. These statements may well apply to one of the most extensively studied ecological patterns, relationships between total phosphorus (TP) and chlorophyll *a* (Chl).

Decades of empirical research have identified several variables that are related to algal biomass in north temperate lake ecosystems (reviewed in Smith 1990 and Mazumder 1994). One of these relationships is the pattern of a consistently lower Chl yields per unit concentrations of nutrients (phosphorus or nitrogen) observed among the large-*Daphnia*-dominated ecosystems in north temperate (Shapiro 1980; Sarnelle 1992; Mazumder 1994), south temperate (Quirós 1990), and antarctic (Hansson 1992) zones compared with systems without large *Daphnia*. These patterns of low Chl yields in lakes having large *Daphnia* appear to be quite robust (Mazumder 1994).

In this paper, I investigate potential mechanisms associated with the observed patterns of TP–Chl relationships in ecosystems with and without large herbivores such as large *Daphnia* (see Mazumder 1994 for details). I use data from controlled experiments in lake enclosures because the initial environmental (physical, chemical, and biological) conditions are constant in all treatments; hence the observed differences in TP–Chl relationships or the patterns of Chl yields may be interpreted as the probable consequences of the experimental manipulations. Specifically, I test the hypothesis that the contributions of dissolved (TDP) and zooplankton phosphorus (ZP) to TP vary significantly between the ecosystems containing contrasting abundances of large *Daphnia* and that these variable contributions of TDP and ZP may be responsible for generating variable Chl yields. In this paper, I emphasize stratified systems because such detailed

TABLE 1. Abundance and mean length of *Daphnia*, mesozooplankton (>200 μm) filtering rates, flux of P into mesozooplankton (filtering rate per day times the concentration of PP in labelled food, 0.2–20 μm), and [^{32}P]phosphate turnover time in enclosures and lakes with contrasting abundances of large *Daphnia*. All data are spring to summer averages (details available in Mazumder et al. 1988, 1990a, 1990b, 1990c, 1992).

	Control	+F	+N	+NF	Haynes	St. George
Daphnia (>1 mm) abundace (no.·L^{-1})	15.1	0.4	13.0	1.8	22.5	0.7
Daphnia mean length (mm)	1.2	0.7	1.3	0.8	1.1	0.7
Mesozooplankton filtering rate (%·d^{-1})	67	13	146	7.4		
P flux to mesozooplankton (μg P·d^{-1})	2.8	0.8	10.3	1.5		
$^{32}PO_4$ turnover time (min)	11	5	73	6	14	3

data are not available from mixed systems. However, an independent data set from the literature consisting of stratified and mixed lakes is analyzed to further test the hypothesis. Then I examine how grazing by contrasting herbivore communities produces contrasting size distributions of Chl (as an indicator of algal size distribution) at constant external loading of nutrients. As the size distribution of algae seems to be related to the flow of P from the dissolved pool to particulate pool (Vézina 1986; Mazumder et al. 1988, 1990c, 1992), I test the hypothesis that grazers can modify the relative contributions TDP and ZP to TP by changing the intensity of phosphate limitation and partitioning of the TP pool. This hypothesis is based on the observation that when large herbivores are lacking (produced by intense planktivory), grazing loss of edible algae (<20 μm) is low compared with when large herbivores are abundant (Mazumder et al. 1990a). Under low grazing pressure on algae, the dissolved and reactive pool of P is relatively small, phosphate limitation is intense, and most of the added nutrients are assimilated by bacteria and small algae (Mazumder et al. 1988, 1990c, 1992). The contribution of ZP to TP and flux of P from edible algae to zooplankton are small. Consequently, a major portion of the TP pool is represented by plankton 2–20 μm when large herbivores are lacking, while a major portion of the TP pool is represented by zooplankton and dissolved pools when large herbivores are abundant.

Materials and Methods

To examine the potential mechanisms associated with the influence of contrasting herbivore community structure and nutrient loading on TP–Chl relationships, I use two years of seasonal data from experiments in large enclosures where additions of planktivorous fish and/or nutrients (P and N) produced contrasting zooplankton community structures. The size distribution of TP, Chl, and zooplankton and processes such as zooplankton grazing and [^{32}P]phosphate turnover times were measured simultaneously (Table 1). Similar data (except grazing) were also collected from two mesoeutrophic lakes with contrasting abundances of large *Daphnia*. Both enclosures and lakes were used as examples of stratified mesoeutrophic systems.

Enclosure experiments (all conducted in Lake St. George, Ontario) have been described in Mazumder et al. (1988). Briefly, planktivorous fish (0+ yellow perch (*Perca flavescens*)) and/or nutrients ($NaNO_3$ and H_3PO_4) were added to eight large enclosures (8 m in diameter, 15 m deep, and open to the sediment) in a 2 × 2 factorial design. Treatments were (1) no fish and no nutrients (control), (2) with fish and no nutrients (+F), (3) no fish and with nutrients (+N), and (4) with fish and with nutrients (+NF). Each treatment had two replicate enclosures.

Two lakes included in this study are Haynes Lake and Lake St. George. These lakes indicate strong thermal stratification during summer. Haynes Lake and Lake St. George are small mesoeutrophic lakes that have been described in Mazumder et al. (1990b) and Lafontaine and McQueen (1991). Haynes Lake had few planktivorous fish (<100·ha^{-1}) and abundant large *Daphnia* (Mazumder et al. 1990b; Lafontaine and McQueen 1991). Lake St. George had abundant planktivorous fish (5000–7000·ha^{-1}) and the zooplankton community is dominated by rotifers and cyclopoid copepods. Although *Daphnia* appear in Lake St. George during summer months, large *Daphnia* are rarely encountered (Table 1).

Data on the size distribution of TP and Chl were collected from the epilimnion (0–3 m integrated samples) five times during spring through summer from enclosures and the two lakes. The size distribution of TP (<0.2, 0.2–1, 1–20, 20–200, and >200 μm; see Mazumder et al. 1988 for methods) was measured following filter-fractionation: the <0.2 μm fraction represents TDP and the >200 μm fraction represents particulate phosphorous (PP) in zooplankton. As the concentration of Chl >64 μm was undetectable, it was considered that TP > 200 μm was largely zooplanktonic. I use TDP and PP > 200 μm (ZP) as the two major heterotrophic or nonautotrophic fractions of TP. I realize that my method of calculating heterotrophic fraction of TP ignores the contributions of ciliates and rotifers which overlap in sizes with nano- (<20 μm) and microplanktonic (>20 μm) algae. A complete separation of autotrophs from heterotrophs can only be done by direct enumeration of the entire plankton community (bacteria to zooplankton), a task which was beyond my capability. Therefore, in this paper, I do not use the remaining fractions (PP = 0.2–1, 1–20, and 20–200 μm) as indicators of autotrophic P. Nevertheless, the fractions of TP used to indicate the possible contributions of nonautotrophic fraction P to TP may be robust enough for supporting my interpretations in this paper. I have also analyzed a limited data set from the literature to examine the relative contributions of TDP to TP and their relationships with Chl yields per unit TP in meso- to hypereutrophic lake ecosystems having contrasting abundances of large *Daphnia* communities (Faafeng and Brabrand 1990; Reinertsen et al. 1990; Sanni and Wærvågen 1990; van Donk et al. 1990).

Results and Discussion

Enclosures and lakes with abundant large *Daphnia* have less than half the Chl yields per unit TP than those lacking large *Daphnia* (Fig. 1). The same nutrient additions

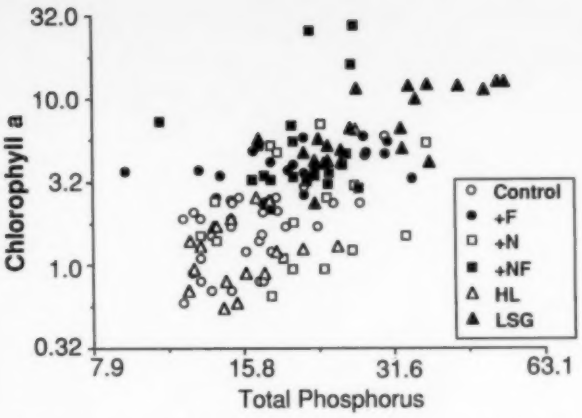

FIG. 1. Relationship between TP ($\mu g \cdot L^{-1}$) and Chl ($\mu g \cdot L^{-1}$). Data are individual dates (biweekly to monthly) during April–August. Treatments in enclosures (control, with fish as +F, with nutrients and without fish as +N, and with nutrients and fish as +NF) are described in the text. Haynes Lake (HL) and Lake St. George (LSG) are with and without abundant large *Daphnia*, respectively. Solid symbols indicate systems lacking large *Daphnia* and open symbols indicate systems with abundant large *Daphnia*.

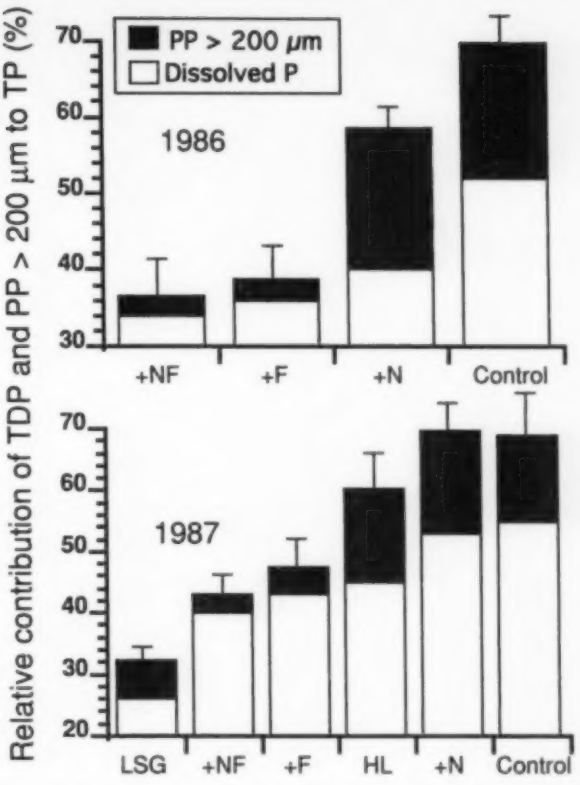

FIG. 2. Relative contribution of P in dissolved (<0.2 μm) and zooplankton (PP > 200 μm) pools (% of TP) among experimental enclosures and two lakes (same as Fig. 1). Error bars are 1 SE of the seasonal means.

(1 g $P \cdot m^{-2} \cdot yr^{-1}$, 13 g $N \cdot m^{-2} \cdot yr^{-1}$) produce higher Chl yields in the enclosures where large *Daphnia* are eliminated by planktivorous fish (+NF) than in the fish-free enclosures where large *Daphnia* are abundant. During the two years of enclosure experiments, the mean concentrations of TP in the +NF enclosures are either slightly lower (15% lower in 1986) than or the same (1987) as those in the +N enclosures. These similar TP concentrations but significantly lower Chl yields in the +N enclosures than in +NF enclosures clearly illustrate a lower algal response to the same nutrient additions when large *Daphnia* are abundant. The low Chl yields are also observed in the unenriched enclosures (control) and Haynes Lake having abundant large *Daphnia*. These patterns of lower Chl yields to TP in the systems having large *Daphnia* are consistent with the patterns observed among a large number of north temperate lake ecosystems with widely variable nutrient concentrations (Mazumder 1994). Others have also observed similar patterns of lower algal biomass at constant TP in lakes having large herbivorous zooplankton (Shapiro 1979, 1980; Hansson 1992; Sarnelle 1992).

Grazer-Induced Modification of P Partitioning

Now that I have shown significantly lower Chl yields per unit TP in the enclosures and lakes having abundant large *Daphnia* than those lacking large *Daphnia*, I examine the characteristics of TP in terms of its size distribution in these contrasting systems. In the enclosures, when large *Daphnia* are abundant, TDP and ZP account for 60–70% of TP (control and +N) compared with 30–50% of TP when large *Daphnia* are eliminated by planktivorous fish (Fig. 2). The contributions of ZP were less than 5% of TP in the enclosures lacking large *Daphnia* (+F and +NF), while they were 15–20% of TP in the enclosures having abundant large *Daphnia* (control and +N). Similarly, in Lake St. George lacking large *Daphnia*, TDP and ZP contribute 26 and 6% to TP, respectively. In Haynes Lake with abundant large *Daphnia*, TDP and ZP contributions to TP are 44 and 18%, respectively. Results on the relative contributions of TDP and ZP in enclosures and lakes with contrasting communities of large herbivores indicate that the heterotrophic fractions of P are relatively more important in the enclosures and lakes (systems) having large herbivores. These systems with large herbivores exhibit lower Chl yields to TP (Fig. 1), an observation consistent with my hypothesis that systems with large herbivores show lower Chl:TP ratios than systems where large herbivores are lacking because heterotrophic fractions of P (TDP and ZP) contribute relatively more to TP.

My hypothesis that a disproportional contribution of TDP to TP may be partly responsible for producing variable Chl yields to TP (micrograms Chl per microgram TP) among systems with contrasting large *Daphnia* communities, is further tested by comparing the relative contribution of TDP to TP with the Chl yields per unit TP (Fig. 3). In stratified systems, low Chl yields in the systems having abundant large *Daphnia* are associated with the high contributions of TDP to TP (Fig. 3, top panels). Similar relationships are also observed when within lake, but between-year changes in the relative contributions of TDP to TP are compared with Chl yields (Fig. 3, middle and bottom panels). In each of these four lakes, regardless of TP concentrations or of the presence (Lake Haugatjern) or absence (Lakes Mosvatn,

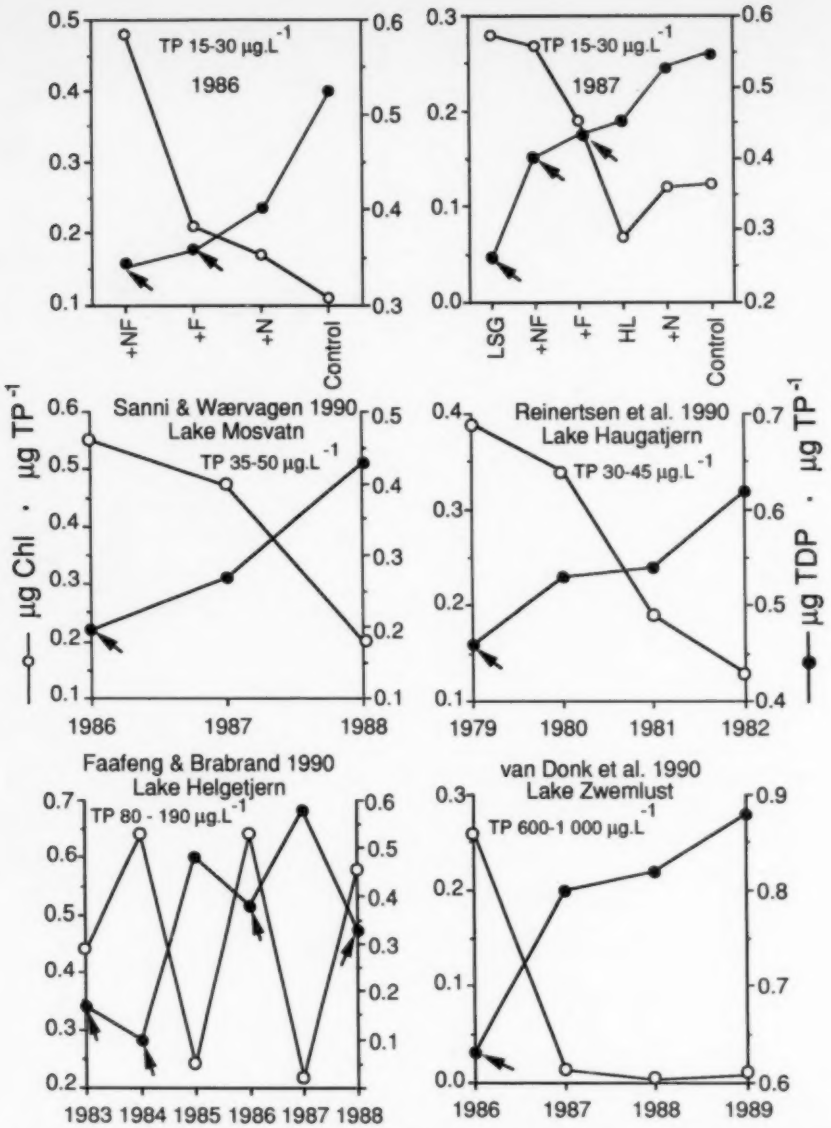

FIG. 3. Relative contribution (May –August seasonal mean) of TDP to TP and the concentrations of Chl per unit TP. Top panels: among experimental enclosures and two lakes with contrasting large *Daphnia* communities; middle panels: between-year differences within each of the two eutrophic lakes; bottom panels: between-year differences within each of the two highly hypereutrophic lakes. Arrows indicate the systems or years within systems lacking large *Daphnia*.

Helgetjern, and Zwenlust) of thermal stratification, the presence of abundant large *Daphnia* is associated with the higher contributions of TDP to TP and lower Chl yields.

To examine the consistency of the relationship that a greater contribution of TDP to TP is associated with lower Chl yields per unit TP, data on the relative contributions of TDP to TP are plotted against the Chl yields per unit TP (Fig. 4). In this data set, the Chl yields per unit TP vary from 0.002 to 1 µg Chl·µg TP^{-1} and the contributions of TDP vary from 0.05 to 0.95 µg TDP·µg TP^{-1}. The observed relationship illustrates that the contributions of TDP to TP decline with increasing yields of Chl per unit TP. As the two variables used in the comparison use a common denominator (TP), I do not develop a predictive model for this relationship. However, the observed patterns appear to be sufficiently robust to suggest that the variable contributions of TDP to TP are important in generating highly variable Chl yields per unit TP under contrasting abundances of large *Daphnia*. Lower contributions of soluble reactive phosphorous (SRP) and TDP in systems with abundant large herbivores have also been observed by others (Shapiro 1980; Hansson 1992). The patterns of TP–Chl relationships pro-

duced by a wide range of north temperate aquatic ecosystems indicated that TP is a stronger predictor of Chl yields (indicated by r^2) when large herbivores are lacking than when they are present (Mazumder 1994). I suggest that when large grazers are lacking, TP tends to approximate Chl yields more closely because a major fraction of P may be tied up in algal communities.

Size Distribution of Chl under Contrasting Herbivory

Other important mechanisms of how grazers may modify the partitioning of P and the resultant TP–Chl relationships probably concern the size distribution of algae (measured as Chl) and associated dynamics of available P. The concentration of small algae, especially the pico- and nanoplanktonic algae (Chl < 20 μm), is reduced substantially when large *Daphnia* dominate the zooplankton communities (Fig. 5). In the fish-free enclosures where large *Daphnia* are abundant (control), 30% of the Chl is > 20 μm compared with 15% in +F enclosures where large *Daphnia* are eliminated by the planktivorous fish. In Haynes Lake and Lake St. George, 40 and 5% of the Chl is >20 μm, respectively. These results indicate that small pico- and nanoplanktonic algae dominate the algal communities when large *Daphnia* are eliminated by planktivorous fish.

Addition of nutrients further increases the contribution of Chl > 20 μm only when large *Daphnia* are abundant (50% of Chl > 20 μm in +N enclosures). In the +NF enclosures where large *Daphnia* are virtually absent, addition of nutrients increases the concentration of pico- and nanoplanktonic algae (95% of Chl < 20 μm) rather than that of microalgae (Chl > 20 μm). The concentrations of Chl > 20 μm or >30 μm are often used to indicate the fraction of algae that may be less edible by *Daphnia* (Porter 1972; Gliwicz 1977; McCauley and Briand 1979; Leibold 1990; Mazumder et al. 1990a). Earlier studies (Watson and Kalff 1981; McCauley et al. 1988; Watson and McCauley 1988; Leibold 1990; Watson et al. 1992) have suggested that the contribution of inedible algae increases with enrichment. The data presented in this paper indicate that in mesoeutrophic systems (15–30 μg TP·L^{-1}), enrichment tends to increase the contributions of Chl > 20 μm to total Chl only when large *Daphnia* are abundant, and it tends to increase Chl < 20 μm rather than the Chl > 20 μm when large grazers are removed. If we consider the competitive superiority of small algal cells over large cells in assimilating available nutrients, the observed dominance of microalgae in the large-*Daphnia*-dominated enclosures seems logical (discussed below).

Potential Mechanisms of Grazer and Nutrient Effects Producing Variable Chl Yields

I have shown that abundant large *Daphnia* produce low Chl yields per unit TP (Fig. 1) and that a greater contribution of TDP and ZP to TP may be responsible for producing lower Chl yields in systems with large *Daphnia* compared with systems without large *Daphnia* (Fig. 2). My results have also indicated that there is a consistent pattern of increasing contribution of TDP to TP with decreasing Chl yields per unit TP (Fig. 3 and 4) among mesoeutrophic to hypereutrophic systems. In addition, the pattern of the lower contributions of pico- and nanoplanktonic algae (indicated by Chl < 20 μm) to the total algal biomass in the systems having large *Daphnia* seems to be associated with the lower

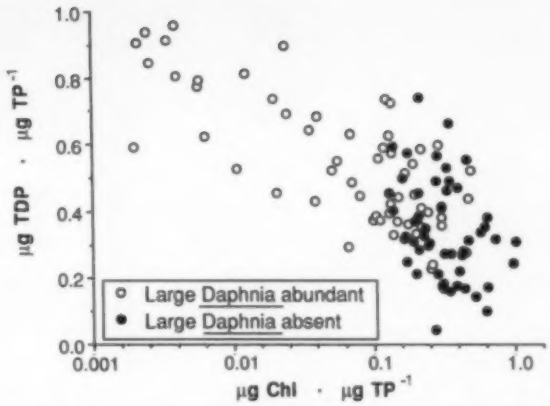

FIG. 4. Relationship between the relative contribution of TDP to TP and the concentration of Chl per unit TP among experimental enclosures and lakes. The data set contains the same data presented in Fig. 3 plus one other study (Tátrai et al. 1990). Data points are individual dates rather than seasonal means.

Chl yields per unit TP (Fig. 5). I now address the potential mechanisms of how the presence and absence of large *Daphnia* and associated grazing could modify the pathways of available P and produce the variable Chl yields.

In fish-free enclosures (control and +N), the high filtering rates by the zooplankton communities on pico- and nanoplankton (1–20 μm; Mazumder et al. 1990a; Table 1) are associated with low concentrations of pico- and nanoplanktonic P (PP = 1–20 μm; Mazumder et al. 1988, 1992) and Chl = 1.2–20 μm (Fig. 5). In these enclosures with abundant large *Daphnia*, a greater flux of nano- and picoplanktonic P to zooplankton via grazing indicates that more autotrophic P appears to be transformed into heterotrophic P (Table 1). When large grazers are not abundant, small algae are abundant, and less of the P bound in algae appears to be transformed into zooplankton P via grazing. In addition, when large *Daphnia* are absent the higher concentrations small algae are associated with a faster turnover time of PO_4 compared with the large *Daphnia* are abundant and the concentrations of small algae are low.

Based on the contrasts in grazing and associated contrasts in the concentrations of small algae and in PO_4 turnover time, I suggest that the contrasting large *Daphnia* may produce variable Chl yields directly by transforming algal P into zooplankton P and indirectly by modifying the competition among the different sizes of algal cells for available P. A faster turnover time of phosphate indicates that the supply of available P is relatively smaller than the demand by the plankton community. When phosphate demand is intense, small cells have an added advantage over large cells because small cells provide greater surface to volume than large cells (Vézina 1986; Mazumder et al. 1988). Therefore, a reduced grazing loss of small algal P to zooplankton, as happens when large grazers are absent, and a faster phosphate turnover time tend to favor more efficient sequestering of P in small algal cells rather than in the dissolved pool and in the zooplankton. The resultant effect should be a greater Chl yield to TP because most of the TP may be tied up in the algae. Systems lacking large *Daphnia* have as high as 0.95 μg Chl·μg TP^{-1} which may mean that most

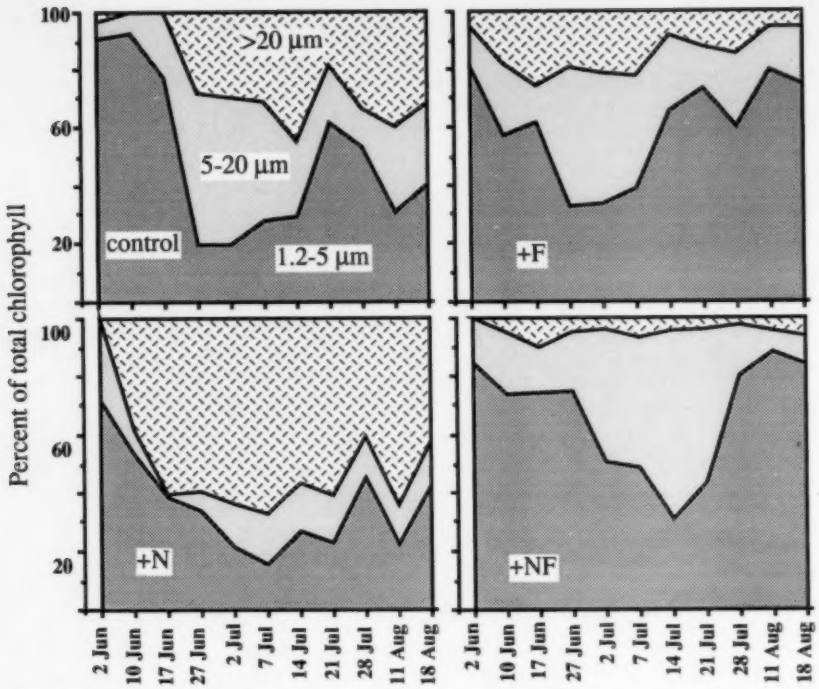

FIG. 5. Size distribution of Chl in three size-classes measured during the 1987 field season. Treatments are same as in Fig. 1.

of the measured P is autotrophic (Fig. 4). Conversely, an increased grazing loss of algal P to zooplankton, as happens when large grazers are abundant, and a longer phosphate turnover time may tend to favor a greater contribution of both TDP and ZP to TP. The resultant effect should be a smaller Chl yield to TP because a large fraction of the TP is tied up in fractions (TDP and ZP) that do not contain Chl. Systems having abundant large *Daphnia* have as low as 0.002 µg Chl·µg TP^{-1} which may mean that very little of the measured P is autotrophic (Fig. 4). Overall, the results presented in this paper show a strong link of the contributions of heterotrophic components of TP with Chl yields per unit TP.

Based on the results presented in the paper, it may be concluded that the variable patterns of Chl yields and associated TP–Chl relationships appear to be generated by a variable partitioning of P in algal versus nonalgal components of TP in systems having contrasting abundances of large *Daphnia*. Let us consider two hypothetical systems with same TP concentrations (20 µg $TP{\cdot}L^{-1}$) but in one system, 70% of TP is represented by zooplankton and dissolved fractions and in another system, 30% of TP is represented by these two fractions. Therefore, in the first system, algae may make up to 30% of the remaining TP (6 µg $TP{\cdot}L^{-1}$), while in the second system, algae may make up to 70% of the remaining TP (14 µg $TP{\cdot}L^{-1}$). If we assumed a 1:1 ratio of Chl:TP in these remaining fractions, then the former system should exhibit a much lower Chl yield per unit TP than the latter system. However, more detailed investigations of the contributions of autotrophic versus herterotrophic P as a function of grazing and nutrient enrichment will be needed to verify the mechanisms proposed in the paper. This approach is presently being investigated by examining the contributions of bacteria, ciliates, autotrophic and heterotrophic flagellates, algae, and all zooplankton groups to total plankton biomass and their relationships with the Chl yields to TP.

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Maturity and Molt Status of Crustacea: Determining Classification without Prior Criteria

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Existing methodology for estimation is reviewed for the situation where, a priori, the existence of two or more groups can be postulated but, in contrast with discriminant analysis, there is no sample in which the correct categories are known. Such mixture models are applied to two data sets related to the maturity and molt status of snow crab, *Chionoecetes opilio*, namely (1) the classification of crab as morphometrically mature or immature on the basis of the chela height – carapace width relationship and (2) the determination of the number of molts of crab, during a known period at liberty, from the relationship of size at release and size at recapture of tagged animals. In the latter example, a theoretical constraint is imposed that links the relationships at the different stages. The solution is obtained by "nesting" an iterative procedure within an EM algorithm. The method permits hypotheses concerning the number of groups to be tested, including the hypothesis that the data come from a single homogenous group, and each individual is assigned a probability of belonging to a group.

Nous examinons la méthodologie d'estimation pour une situation où, a priori, on peut poser qu'il existe au moins deux groupes mais où, contrairement à l'analyse discriminante, il n'existe pas d'échantillon dans lequel on repère correctement les catégories. Ces modèles de mélange sont appliqués à deux séries de données concernant la maturité et la mue du crabe des neiges, *Chionoecetes opilio*, et plus précisément 1) la classification du crabe comme mature ou immature sur le plan morphométrique à partir de la relation hauteur des chélipèdes – largeur de la carapace et 2) la détermination du nombre de mues du crabe, pendant une période de liberté connue, à partir de la relation entre la taille à la libération et la taille à la recapture des animaux marqués. Dans ce dernier exemple, on impose une constrainte théorique qui lie les relations aux différents stades. La solution est obtenue par « imbrication » d'une procédure itérative dans un algorithme EM. La méthode permet de formuler des hypothèses concernant le nombre de groupes à tester, et notamment l'hypothèse selon laquelle les données proviendraient d'un seul groupe homogène, et on assigne à chaque individu une probabilité d'appartenance à un groupe.

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In traditional discriminant analysis, a discriminant function, determined from data for which the correct category of each individual is known, is used to assign individuals of unknown origin to the appropriate category. There are, however, situations where the existence of two or more groups can be postulated but one does not have available a sample in which the correct categories are known and, thus, discriminant analysis is not applicable. Two examples of this nature in fisheries research are as follows.

First, consider the classification of snow crab, *Chionoecetes opilio*, as "mature" or "immature" on the basis of the cela height – carapace width relationship (e.g., Conan and Comeau 1986). The term "mature" here refers to morphometric maturity, objectively described as large-clawed as opposed to small-clawed animals, and does not necessarily coincide with physiological or functional maturity. Conan and Comeau (1986) cited Tessier (1933, 1935) and Hartnoll (1963) as showing that male majid crab show a differentiation of the chelae, a change in allometry, when they reach maturity. There is a discontinuity caused by the pubertal molt or molt to maturity during which the chelae increase considerably in volume in one abrupt step. However, the molt to maturity may occur over a large range of carapace size. Conan and Comeau (1986) developed a discriminant function for classifying snow crab as morphometrically mature or immature on the basis of a "correct" state as determined by the principal components of six morphological measures. From these, they selected carapace width and chela height on which to develop a conventional discriminant function. They achieved a 98% correct classification. There is nothing in their paper to suggest that morphological maturity, on the individuals used for constructing the discriminant function, was determined by any means other than the principal component analysis. Thus, the high rate of correct classification primarily reflects the fact that the variables used in the discriminant function are a subset of the variables used for the construction of the principal components. Had all six variables been used for a discriminant function, a 100% "correct" classification would have resulted. The same procedure was followed by Sainte-Marie and Hazel (1992). In contrast, Donaldson and Johnson (1988) made an a priori classification of the individual *Chionoecetes bairdi* according to whether they were mature male mating crab, as verified by successful fertilization of primiparous female egg clutches, or were from a growth study in which the crab were thought to be primarily morphometrically immature.

Second, consider the determination of the number of molts of lobster, during a known period at liberty, from the relationship between the size at release and the size at recapture (e.g., Conan and Gundersen 1979; Taylor and Hoening 1990). Taylor and Hoening (1990) supported their grouping on the grounds that those presumed to have molted twice were at liberty on average for twice as long as those presumed to have molted once and that the regression of recapture size on size at tagging, for the presumed single-molters, could be used to predict, with reasonable accuracy, the regression for the presumed double-molters. Although Conan and Gundersen's (1979) description of their procedure is in parts unclear, they also began by estimating the regression of carapace width at time of recapture on that at time of release for crab judged to be one-molters on the basis of their time at liberty. The regressions for n-molters are similarly projected. Conan and Gundersen (1979) did not, however, theoretically project the variances about these regressions but attempted their estimation by simulation. Classification is then achieved by examining the differences between the actual carapace width and the expected values from the projected regressions in relation to the simulated variances. The properties of this procedure are unknown.

In these and similar examples the existence of clusters can certainly be hypothesized and often detected by plots of the data, although the boundaries between such clusters may be poorly demarcated. In this paper the methodology for objectively classifying individuals is applied to data representative of the above two examples. Although the methodology is, for the most part, well established in the statistical literature, and there exist many examples of its application (e.g., Aitkin et al. 1981; De Veaux 1989; Mclaren et al. 1991; Boos and Brownie 1991; in particular, McLachlan and Basford 1988), it seems virtually unknown for fisheries applications. For completeness, therefore, the methodology is first presented.

Methodology

To begin, suppose that there is reason to believe that a particular population is made up of two subpopulations. In particular, suppose that $\mathbf{y}_i$, $i = 1, 2, \ldots, n$ is a vector of k measurements made on the ith individual and can be regarded as random observation from a multivariate distribution with parameter $\boldsymbol{\theta}_1$ or $\boldsymbol{\theta}_2$, according to whether it belongs to the first or second subpopulation. For example, for a bivariate measure ($k = 2$), we may think of $\boldsymbol{\theta}$ as the marginal means, variances, and covariance (or correlation) of a bivariate normal (or lognormal) distribution. Let $f_j(\mathbf{y})$ denote the density function for the jth subpopulation, $j = 1, 2$. Further, let the proportion in the first subpopulation be p. The likelihood of the observed sample is then

$$L = \prod_{i=1}^{n} [\, p f_1(\mathbf{y}_i) + (1-p) f_2(\mathbf{y}_i)].$$

The task now is to obtain the maximum likelihood estimates of the $\boldsymbol{\theta}_j$ and p. In the case of a bivariate normal model, there is a total of 5 + 5 + 1 = 11 parameters.

Multivariate Normal Model with Two Groups

Explicit expressions for the estimates in the case of the multivariate normal distribution, with common variance–covariance matrix, have been given by Day (1969). Let $\boldsymbol{\mu}_j$ denote the mean vector in the jth subpopulation and let Σ denote the (common) variance–covariance matrix. The likelihood may then be written

$$(2\pi)^{-nk/2} |\Sigma|^{-n/2} \prod_{i=1}^{n} [\, p e^{-Q_{1i}/2} + (1-p) e^{-Q_{2i}/2}]$$

where

$$Q_{ji} = (\mathbf{y}_i - \boldsymbol{\mu}_j)' \Sigma^{-1} (\mathbf{y}_i - \boldsymbol{\mu}_j).$$

Let $P(j|i)$ denote the probability that observation $\mathbf{y}_i$ belongs to the jth subpopulation. Thus:

$$\hat{P}(1|i) = \hat{p}\hat{f}_1(\mathbf{y}_i) / [\, \hat{p}\hat{f}_1(\mathbf{y}_i) + (1-\hat{p})\hat{f}_2(\mathbf{y}_i)]$$

where the "hats" indicate estimates. Then, as demonstrated by Day (1969), the conventional maximum likelihood equations, obtained by equating to zero the partial differentials of the logarithm of the likelihood with respect to each of the unknown parameters, can be written in the following form:

$$\hat{p} = \sum_i \hat{P}(1|i) / n$$

$$\hat{\boldsymbol{\mu}}_j = \left[\sum_i \mathbf{y}_i \hat{P}(j|i) \right] / \sum_i \hat{P}(j|i)$$

$$\hat{\Sigma} = \left[\sum_j \sum_i (\mathbf{y}_i - \hat{\boldsymbol{\mu}}_j)' (\mathbf{y}_i - \hat{\boldsymbol{\mu}}_j) \hat{P}(j|i) \right] / n.$$

(Note that Day (1969) gives the expression for $\hat{p}$ in an implicit, rather than explicit, form.)

Thus, given any assumed values for the $\hat{P}(j|i)$, one may readily compute estimates of the p, $\boldsymbol{\mu}_j$, and Σ. Conversely, given values for these parameters, one may readily compute the $\hat{f}_j(\mathbf{y}_i)$ and, in turn, estimates of the $P(j|i)$. Dempster et al. (1977) noted that this procedure is a particular case of the EM algorithm. One may start by assuming a potential grouping for the $\mathbf{y}_i$, i.e., one may set the $\hat{P}(j|i)$ to 0 or 1 as seems appropriate. It is convenient but not necessary that these initial values be 0 or 1; the only requirement is that $0 \le \hat{P}(j|i) \le 1$ and $\Sigma_j \hat{P}(j|i) = 1$ for all i. One may then estimate the p, $\boldsymbol{\mu}_j$, and Σ, reestimate the $P(j|i)$, reestimate the p, $\boldsymbol{\mu}$, and Σ, etc. until the convergence is attained. An example, assuming a univariate normal distribution ($k = 1$), is given in Aitkin and Wilson (1980).

The assumption of a common variance–covariance matrix appears rather restrictive. Day (1969) briefly considered the case of unequal variances in the univariate normal model, and Aitken and Wilson (1980) extended their univariate example to the case of unequal variances. (Note that the expression for $\hat{\sigma}_j^2$ given in the 1980 paper is in error; the correction is given in Aitkin and Wilson 1981). The multivariate extension is, however, quite straightforward. The likelihood can be written

$$(2\pi)^{-nk/2} \prod_{i=1}^{n} [\, p e^{-Q_{1i}/2} / |\Sigma_1|^{1/2} + (1-p) e^{-Q_{2i}/2} / |\Sigma_2|^{1/2}]$$

where now

$$Q_{ji} = (\mathbf{y}_i - \boldsymbol{\mu}_j)' \Sigma_j^{-1} (\mathbf{y}_i - \boldsymbol{\mu}_j).$$

The only change in the maximum likelihood equations is that the expression for $\hat{\Sigma}$ is replaced by

$$\hat{\Sigma}_j = \sum_i (\mathbf{y}_i - \hat{\boldsymbol{\mu}}_j)' (\mathbf{y}_i - \hat{\boldsymbol{\mu}}_j) \hat{P}(j|i) / \sum_i \hat{P}(j|i).$$

Day (1969) observed that "each sample point generates a singularity in the likelihood function. Similarly any pair of sample points which are sufficiently close together will generate a local maximum, as will triplets, quadruplets and so on which are sufficiently close . Maximum likelihood clearly breaks down." Aitkin and Wilson (1980), however, while noting the existence of singularities, indicated that "these do not cause any computational difficulty in practice." They also stated that "It is characteristic of mixture and latent variable models that multiple local maxima of the likelihood function may occur" and pointed out that "different starting values (corresponding to different outlier specifications [or, in our context, different initial groupings]) therefore need to be tried." (See also Hathaway 1985; McLachlan and Basford 1988, sections 1.8 and 2.1.) The question of how many sets of initial estimates are necessary to consistently find the global maximum has been addressed by Atwood et al. (1992).

Regression Model for Two Groups

The above has considered the case where the $\boldsymbol{y}_i$ are regarded as random observations from some multivariate distribution (or mixture of distributions). Thus, each component of the $\boldsymbol{y}_i$ is, itself, a random variable. This may not be a proper representation of the situation. For example, one may be interested in discriminating in the case where one measure, y say, is regarded as a function of one or more other variables, $\boldsymbol{x}$, where the latter are not necessarily generated via a random sample. A regression model

$$y_i = \boldsymbol{\beta}\boldsymbol{x}_i + \boldsymbol{\epsilon}_i$$

with the random component, $\boldsymbol{\epsilon}_i$, following some assumed distribution, might then be appropriate. Conventionally the $\boldsymbol{\epsilon}_i$ will be assumed independent, normally distributed with mean zero and variance σ^2. For any given value of $\boldsymbol{x}_i$ the observed y_i may come from one of two populations characterized by different values of $\boldsymbol{\beta}$ and, perhaps, σ^2, namely $\boldsymbol{\beta}_1$, σ_1^2 and $\boldsymbol{\beta}_2$, σ_2^2. As before, let the probability of y_i arising from the first population be p. It is assumed that p is constant, i.e., it does not depend on the $\boldsymbol{x}_i$.

The model probability density function of y is then $pf_1(y) + (1 - p)f_2(y)$ where now

$$f_j(y) = (2\pi\sigma^2)^{-1/2} e^{-(y-\boldsymbol{\beta}_j x)^2/2\sigma^2}$$

in the case of equal variance and

$$f_j(y) = (2\pi\sigma_j^2)^{-1/2} e^{-(y-\boldsymbol{\beta}_j x)^2/2\sigma_j^2}$$

otherwise. The situation is analogous to the univariate case described above with the $\boldsymbol{\beta}_j\boldsymbol{x}$ and σ_j^2 playing the role of the μ_j and Σ_j, respectively.

Aitkin and Wilson (1980) gave the likelihood equations for the case where a regression model applies in one group and a constant mean in the other, and the residual variance is common. They stated that "Generalizations to unequal variances and to more than two components (one using the regression model, the others for "outliers" with different means), are also straightforward.". The case of regression models in all components is equally straightforward; indeed, the case of a constant mean is simply the regression model with $\boldsymbol{x}_i = \boldsymbol{1}$, all i. Specifically,

$$\hat{p} = \sum_i \hat{P}(1|i)/n$$

as before and

$$\boldsymbol{\beta}_j = (X'P_jX)^{-1}X'P_j\boldsymbol{y}$$

where $P_j = \text{diag}(\hat{P}(j|i))$,

$$\hat{\sigma}^2 = \sum_j \sum_i (y_i - \hat{\boldsymbol{\beta}}_j \boldsymbol{x}_i)^2 \hat{P}(j|i)/n$$

in the case of equal variances and

$$\hat{\sigma}_j^2 = \sum_i (y_i - \hat{\boldsymbol{\beta}}_j \boldsymbol{x}_i)^2 \hat{P}(j|i) / \sum_i \hat{P}(j|i)$$

otherwise. $\hat{P}(j|i)$ is again found as

$$\hat{p}\hat{f}_1(y_i)/[\hat{p}\hat{f}_1(y_i) + (1-\hat{p})\hat{f}_2(y_i)].$$

The assumption that p is constant and not dependent on the $\boldsymbol{x}_i$ is open to question. I hope to explore the relaxation of this assumption, particularly with p as a well-defined function of the $\boldsymbol{x}_i$, in a future paper.

As illustrated by Aitkin and Wilson (1980), the extension to more than two groups is straightforward. Let there be m potential groups. Essentially, the only change in any of the above is that p and $1 - p$ are replaced by $p_1, p_2, \ldots, p_m$ with $\Sigma p_i = 1$.

While many of the above formulae are specific to the case of normally distributed variables, the normality assumption is not required for the procedure per se. One needs only to substitute the appropriate distributional form for the $f_j(\cdot)$ and develop the maximum likelihood estimators of its parameters. In practice, this may, of course, not be easy. The lognormal case can be handled by a simple transformation. In other situations, a practical solution might be to achieve approximate normality through a Box–Cox transformation (Box and Cox 1964).

Examples

1. Assigning Morphomotric Maturity Status to Snow Crabs based on the Carapace Width – Chela Height Relationship

The first example uses data on carapace width and chela height of snow crab provided by E. Dawe (Department of Fisheries and Oceans, Science Branch, Northwest Atlantic, Fisheries Centre, St. John's, Nfld.). The data set chosen was typical of the several sets available. The set originally contained 447 points but two of these were so far removed from the body of the data that they had to be regarded as erroneous and were deleted. Similarly to fig. 4a of Conan and Comeau (1986), a plot of the remaining 445 points gave the impression of two clusters that merged at the lower values of carapace width and chela height. These clusters might logically be regarded as corresponding to morphometrically mature (large-clawed) and immature (small-clawed) animals.

Both bivariate normal and regression models were used. Also, since there seemed to be a degree of positive skewness in the marginal distributions, the data were logarithmically transformed to better induce bivariate normality. This transformation was also employed for the regression model

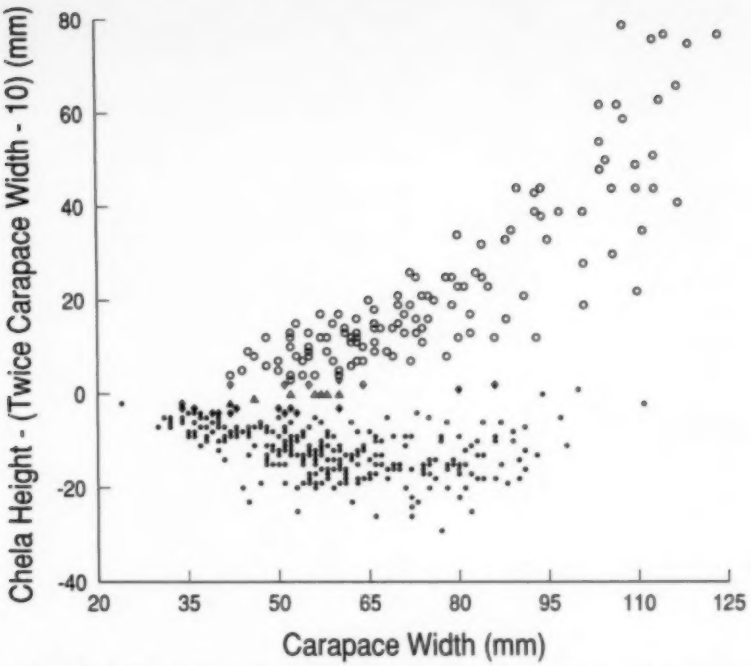

FIG. 1. Plot of chela height minus (twice the carapace width minus 10) against carapace width with the probability of belonging to the first population indicated symbolically. Bivariate lognormal model. Solid circles: $P(1|i) \geq 0.95$; solid diamonds: $0.75 \leq P(1|i) < 0.95$; solid triangles: $0.50 \leq P(1|i) < 0.75$; open triangles: $0.25 < P(1|i) < 0.50$; open diamonds: $0.05 < P(1|i) \leq 0.25$; open circles: $P(1|i) \leq 0.05$.

TABLE 1. Values of the log-likelihood for the bivariate models fitted to the snow crab data. $(\boldsymbol{\mu}, \Sigma)$ refers to the case of a single homogeneous population, $(\boldsymbol{\mu}_1, \boldsymbol{\mu}_2, \Sigma)$ refers to the case of two subpopulations with different means but common variance–covariance matrix, and $(\boldsymbol{\mu}_1, \boldsymbol{\mu}_2, \Sigma_1, \Sigma_2)$ refers to the case of two subpopulations with different means and different variance-covariance matrices.

Model	Log-likelihood
Normal	
$(\boldsymbol{\mu}, \Sigma)$	−2993.4
$(\boldsymbol{\mu}_1, \boldsymbol{\mu}_2, \Sigma)$	−2878.0
$(\boldsymbol{\mu}_1, \boldsymbol{\mu}_2, \Sigma_1, \Sigma_2)$	−2770.6
Lognormal	
$(\boldsymbol{\mu}, \Sigma)$	−2822.7
$(\boldsymbol{\mu}_1, \boldsymbol{\mu}_2, \Sigma)$	−2728.8
$(\boldsymbol{\mu}_1, \boldsymbol{\mu}_2, \Sigma_1, \Sigma_2)$	−2698.4

TABLE 2. Values of the log-likelihood for the regression models fitted to the snow crab data. The coding is as in Table 1 but with $\boldsymbol{\beta}$ and σ^2 replacing $\boldsymbol{\mu}$ and Σ, respectively

Model	Log-likelihood
Normal	
$(\boldsymbol{\beta}, \sigma^2)$	−1454.2
$(\boldsymbol{\beta}_1, \boldsymbol{\beta}_2, \sigma^2)$	−1290.1
$(\boldsymbol{\beta}_1, \boldsymbol{\beta}_2, \sigma_1^2, \sigma_2^2)$	−1262.3
Lognormal	
$(\boldsymbol{\beta}, \sigma^2)$	−1390.0
$(\boldsymbol{\beta}_1, \boldsymbol{\beta}_2, \sigma^2)$	−1208.0
$(\boldsymbol{\beta}_1, \boldsymbol{\beta}_2, \sigma_1^2, \sigma_2^2)$	−1207.5

because there is reason to suppose that the relationship between chela height (y) and carapace width (x) may be better represented by the allometric equation $y = ax^b$ (or $\log y = \log a + b \log x$) than the simple linear model $y = a + bx$ (cf. Conan and Comeau 1986).

The starting values for $P(1|i)$ were taken as 1 or 0 according to whether the ith point fell above or below the major axis of the ellipse of concentration determined under the assumption of a single homogenous bivariate distribution. This implies that $j = 1$ corresponds to the postulated mature (large-clawed) group. (For a definition of the ellipse of concentration, see, for example, Kendall and Buckland 1982.) The use of other starting values showed that the above strategy appeared to lead consistently to convergence to the largest of the local maxima in the interior of the parameter space.

The log-likelihoods under the several bivariate models and the several regression models are given in Tables 1 and 2, respectively.

Aitkin and Wilson (1980) observed that "Since the maximized log-likelihood function can be easily evaluated, it is tempting to formulate likelihood ratio tests for the number

TABLE 3. Frequency of falling into the classes as defined.

	$P(1\|i)$					
Model	≥0.95	0.75–0.95	0.50–0.75	0.25–0.50	0.05–0.25	≤0.05
Normal	105	129	8	9	6	188
Lognormal	234	14	1	6	5	185
Linear	111	82	51	7	11	183
Allometric	181	37	16	12	12	187

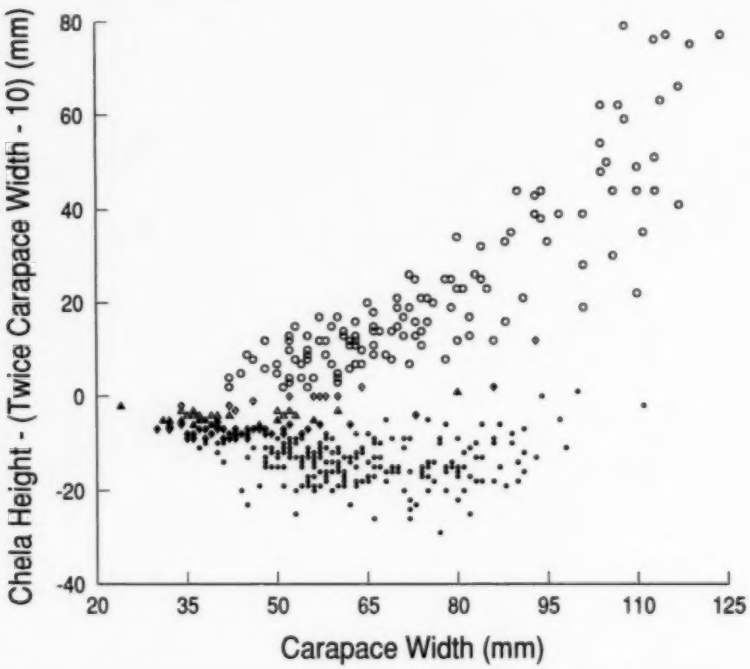

FIG. 2. Plot of chela height minus (twice the carapace width minus 10) against carapace width with the probability of belonging to the first population indicated symbolically. Allometric regression model. Symbols as in Fig. 1.

of components in the mixture, by the usual comparison of the maximized log-likelihoods." They went on to point out that, while for *known p* the likelihood ratio test would have an asymptotic χ^2 distribution, when p is unknown and, thus, estimated, the usual χ^2 test with degrees of freedom based on counting the parameters is not valid. Thode et al. (1988) used simulation to obtain percentage points of the null distribution when the alternative is a mixture of two normals with unknown mean and unknown but common variance. They found the χ^2 approximation to be somewhat liberal, i.e., incorrectly reject at a frequency greater than nominal, although with large samples (>1000) the discrepancy was not large. (See also Royston 1989.) Notwithstanding, the likelihood ratio (or twice the difference between the maximized log-likelihoods) can be used as an approximate test for the number of components. It seems clear that, in the bivariate case, the existence of two subpopulations with different means and variances is well established. A parallel conclusion would be reached for the regression models although, in the allometric case, the improvement gained by assuming different variances is negligible.

The likelihood ratio test requires a "nesting" of models, thus, even in the case of known p, there is no likelihood ratio test for comparing normal versus lognormal models in the bivariate case, or linear versus allometric models in the regression case. Nevertheless, the maximized likelihoods can still be used in an informal way and indicate the greater applicability of lognormal and the allometric models, respectively.

Since the bivariate and regression models are inherently different, there is no basis for comparing the log-likelihoods of Table 1 with those of Table 2. (Note that the log-likelihoods are calculated on the untransformed scale with, as is customary, the common term $-n \log \sqrt{2\pi}$ omitted.) Figures 1 and 2 have been constructed from the $\hat{P}(1|i)$ for the lognormal and allometric models, respectively, under the assumption of unequal variance. (The more parsimonious equal-variance allometric model might be preferred; the difference is, however, inconsequential.) To produce a more compact graph in which the differentiation between the clusters can be more clearly illustrated, the ordinate has been transformed to chela height minus (twice the carapace width minus 10).

This has the effect of having the two clusters separated, approximately, by the carapace-width axis. The number of points that fall into the various classes is given in Table 3. For example, under the normal model, there are 105 points that are assigned a probability of at least 95% of belonging to the first population.

One might anticipate that, for any fixed value of carapace width, $\hat{P}(1|i)$ would be a monotonically increasing function of chela height. While this turns out to be generally the case, exceptions occur under both the bivariate-normal and linear-regression models. Under the latter, these are a consequence of the estimated regression lines intersecting at a carapace width of 39.3 mm and a chela height of 59.4 mm, which is inside the range of the data. With carapace widths less than 39.3 mm the relative position of the regression lines is reversed. However, at carapace widths less than 50.0 mm, only a few of the points can be allocated to one or other of the two clusters with a probability greater than 0.95. The explanation in the case of the bivariate normal model is more subtle. For example, for the second population or lower cluster, the $\hat{P}(2|i)$ will decrease as one moves vertically from the centre of the cluster. Accordingly, at any given carapace width, there is a chela height below which the $\hat{P}(2|i)$ will decrease with decreasing chela height. However, the value cannot fall below 0.50; otherwise the point would be given a higher probability of belonging to the upper cluster.

The same phenomenon is not observed under the allometric-regression and bivariate-lognormal models. In theory, such would still be possible; however, in the former case the regression lines now intersect at carapace width 23.5 mm and chela height 33.3 mm, which is just outside the range of the data. The nonoccurrence in the latter (unequal-variance) case is seemingly related to the variance of chela height given carapace width here increasing with carapace width.

Not only do the lognormal and allometric models appear more appropriate under the likelihood criterion, but they also provide clearer differentiation between the clusters than the bivariate-normal and linear-regression models. The only essential difference between the lognormal and allometric models occurs with the smaller carapace widths. As noted above, the allometric model generates two regressions that intersect just outside the range of the data. Consequently, it is virtually impossible to allocate crab with small carapace width (<50 mm, say) to one or the other category. On the other hand, the bivariate lognormal model generates two clusters with comparatively less "overlap" in the small chela-height range (Table 3). The question of which is the more appropriate model is addressed in the Discussion.

2. Estimating the Number of Molts for Recaptured Snow Crabs

The data for the second example were provided by Taylor and Hoenig (1990) and consist of carapace width at tagging and at recapture of 757 snow crab from Conception Bay, Newfoundland. These are presented (Fig. 3) as a Hiatt growth diagram (Hiatt 1948) in which the size at recapture is plotted against the size at release; because the vast majority of the data showed no increment (623 of the 757 cases), points with carapace width at recapture equal to that at release are omitted from the plot. On the basis of the increment in carapace width, Taylor and Hoenig (1990) (aided by the time at liberty) classified these crab as having molted once or twice or as not having molted, i.e., three categories. Most of those classified as not having molted showed no change in carapace width. Some, however, exhibited a small increase (up to 5 mm) in width and others a decrease. The latter, in particular, were attributed to measurement error. The present objective is to reclassify these data through use of the methods described above.

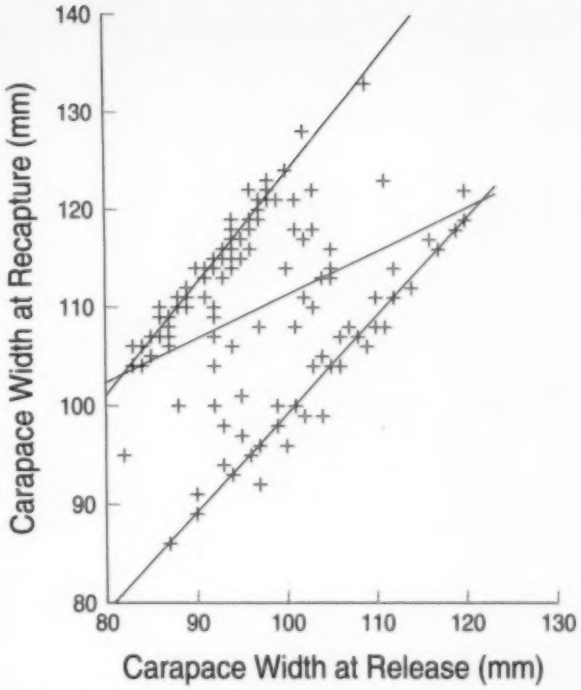

FIG. 3. Plot of carapace width at recapture against carapace width at release with the regression lines as estimated without the constraint.

Because the data comprise initial and final measurements, the regression model would appear to be the more appropriate. Further, the assumption of common variance seems untenable. The likelihood is then

$$L = \prod_{i=1}^{n} \sum_{j=1}^{3} p_j f_j(y_i)$$

where

$$f_j(y_i) = (1/\sqrt{2\pi}\sigma_j)\exp[-(y_i - a_j - b_j x_i)^2 / 2\sigma_j^2]$$

$$p_1 + p_2 + p_3 = 1$$

and the x_i and y_i are the size at tagging and recapture, respectively.

With this model and these data the method leads to a pathological solution. This stems from the vast majority of the data showing no increment, i.e., falling on the line $y = x$. Allowing for unequal σ_j^2 then results in the following three groups (i) those that would be classified as two-molters, (ii) those falling on the line $y = x$ with $\sigma_j^2 = 0$, and (iii) those that would be classified as one-molters along with the remaining nonmolters, distributed with high variance about a line that corresponds to neither one group nor the other.

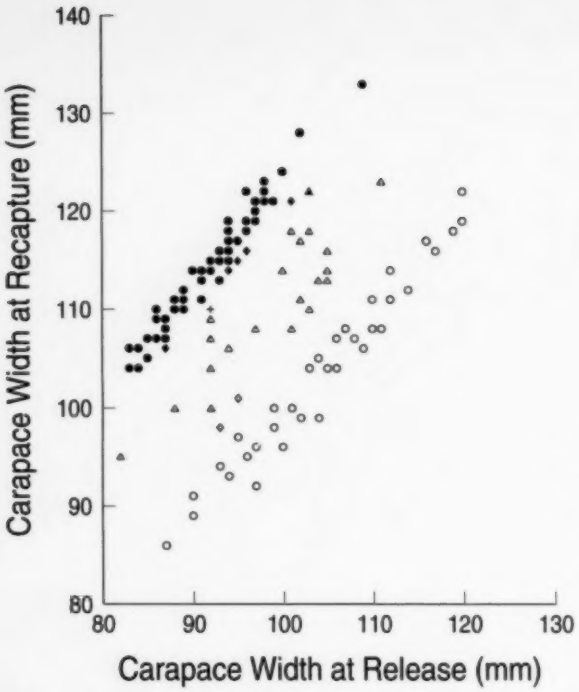

FIG. 4. Plot of carapace width at recapture against carapace width at release with the probability of belonging to a molt stage indicate symbolically. Open circles: $P(1|i) \geq 0.95$; open diamonds: $0.5 < P(2|i) < 0.95$; open triangles: $P(2|i) \geq 0.95$; plus signs $0.5 < P(2|i) < 0.75$; solid triangles: $0.5 \leq P(3|i) < 0.75$; solid diamonds: $0.75 \leq P(3|i) < 0.95$; solid circles: $P(3|i) \geq 0.95$.

The analogous phenomenon occurs if the problem is reduced to a univariate form, i.e., the data are transformed to the differences $y_i - x_i$. It does not occur if one imposes the restriction of equal variance but, as noted above, such restriction is not compatible with the nature of these data.

One might anticipate that the situation would be remedied by removing the 623 cases of no growth (clearly no-molters) from the data set. The result of applying the general regression to the remaining 134 points still appears inappropriate (Fig. 3). As anticipated, the remaining no-molters and the two-molters turn out to be well defined by two almost parallel lines of almost unit slope. The other group (one-molters) is defined by a line of slope 0.458 combined with a high residual variance. The result is that neither crab with smaller initial carapace width and an increment that logically would cause them to be classified as two-molters nor crab with larger initial carapace width and an increment that would logically cause them to be classified as no-molters can be distinguished from one-molters. This stems from the larger variance, and thus poorer definition, apparent with the middle group (one-molters) and the failure to use all the prior information available.

Specifically, let x be the initial size and $y1$ the size after one molt and assume, as above, $y1 = a + bx$. Then the size after two molts, $y2$, should be given by $y2 = a + b(a + bx) = a(b + 1) + b^2x$ (Kurata 1962; cf. Conan and Gundersen 1979). For the no-molters, one should have, in expectation, $y = x$. Accordingly the $f_j(y_i)$ of the above likelihood can be revised to

$$f_1(y_i) = (1/\sqrt{2\pi}\sigma_1)\exp[-(y_i - x_i)^2/2\sigma_1^2]$$

$$f_2(y_i) = (1/\sqrt{2\pi}\sigma_2)\exp[-(y_i - a - bx_i)^2/2\sigma_2^2]$$

$$f_3(y_i) = (1/\sqrt{2\pi}\sigma_3)\exp[-(y_i - a(b+1) - b^2x_i)^2/2\sigma_3^2].$$

As shown in the Appendix, the likelihood equations for a and b can be rewritten with a as subject as

$$a = \frac{\Sigma_i[\pi_{2i}(y_i - \hat{y}_i) + \pi_{3i}(b+1)(\hat{y}_i - b\hat{y}_i)]}{(b+1)\Sigma_i\pi_{3i}}$$

$$a = \frac{\Sigma_i[\pi_{2i}x_i(y_i - \hat{y}_i) + \pi_{3i}(\hat{y}_i + bx_i)(y_i - \hat{y}_i)]}{\Sigma_i\pi_{3i}(\hat{y}_i + bx_i)}$$

where $\pi_{ji} = P(j|i)/\sigma_j^2$. In spite of their appearance, the right-hand sides of the above are functions of a as well as b. We start the system with an assumed set of $\hat{P}(j|i)$. However, unlike the previous example, and the unrestricted regression model, where the unknown parameters could be written explicitly in terms of the data and the $\hat{P}(j|i)$, one now has to imbed an iterative procedure for obtaining $\hat{a}$ and $\hat{b}$ within the EM algorithm. Let us take initial values for a and b, say a_0, b_0. The above equations can be used to compute, for the given b, new values for a, say a_1 and a_2. Now, unless we have been remarkably lucky, a_1 (and a_2) will differ from a_0. Heuristically, one can suppose that the value of a that solves the first equation will lie between a_0 and a_1. Let us replace a_0 by $(a_0 + a_1)/2$ and repeat the process. It is found that this procedure rapidly converges to the solution of the first equation, given $b = b_0$. Let a_1 now denote this solution. In a parallel manner the solution a_2 for the second equation can be obtained, again for $b = b_0$. Unless we have selected the correct value of b, $a_1 \neq a_2$.

Select a new value for b, say b_1, and repeat. If the difference between a_1 and a_2 moves closer to zero, while maintaining the same sign, b has been moved in the right direction. Conversely, if the difference between a_1 and a_2 keeps the same sign but increases, the b has been moved in the wrong direction. If the sign changes, the solution for b must lie between b_0 and b_1. Once two values of b have been found such that the $a_1 - a_2$ take on opposite signs, it is straightforward to converge to the value of b for which $a_1 - a_2 = 0$ and thus, the solution of the likelihood equations.

With these estimates of a, b, and the p_j, and σ_j^2 the EM algorithm can proceed, i.e., the values of $\hat{P}(j|i)$ can be revised and the process repeated until convergence is attained. For the present example, this took 16 iterations.

The system was initiated with groupings as determined by Taylor and Hoenig (1990) ($\hat{P}(j|i) = 0$ or 1). The data file provided was, however, not their final version and contained instances that they later judged to be misclassifications. No corrections were made because it was of interest to see how the method would handle this. The misclassified individuals were allocated, with high probability, to their correct groups on the first iteration.

The final parameter estimates were $a = 8.6599$, $b = 1.0264$, $\sigma_1^2 = 3.9456$, $\sigma_2^2 = 16.2627$, $\sigma_3^2 = 2.2095$, $p_1 = 0.2916$, $p_2 = 0.1934$, $p_3 = 0.5150$. Note that these refer to the case for which individuals with $y_i = x_i$ have been omitted. The above values for a and b provide estimates of the intercept and

slope of the relationship for crab that molted once. The relationship for those that molted twice is then

$$y = 17.5484 + 1.0535x.$$

Of the 134 points in the data set, 64, 22, and 39 were allocated with probability greater than 95% to the two-molt, one-molt, and no-molt groups, respectively, i.e., 125 individuals or 93.3%, were allocated to one or another group with better than 95% probability (see Fig. 4). Of the remaining nine points, two were intermediate between the no-molt and one-molt groups but with probabilities favouring the one-molt group (0.93 and 0.71). Seven points fell between the one-molt and two-molt groups. With five of these the probabilities strongly favoured the two-molt group (0.947, 0.943, 0.939, 0.910, and 0.848), one favoured the one-molt group (probability 0.75), and the other was indeterminate (probability of 0.509 in favour of the two-molt group).

The log-likelihood for the restricted model was −286.4. This can be compared with −276.4 for the unrestricted model. For the latter, 12 parameters were estimated in contrast with eight for the former. Thus, if treated as a χ^2 on 4 df, twice the difference between the log-likelihoods appears as highly significant. Nevertheless the restricted model is still far superior to a single-regression model (i.e., no grouping), for which the log-likelihood is −352.6 (three parameters estimated).

The nature of the model suggests that the variation about the regression should increase with the number of molts. However, the estimate of the variance for the one-molt group noticeably exceeds those for the zero- and two-molt groups. It can be speculated that this is in some way related to the times of tagging and recapture in relation to crab development; crab were tagged during several research cruises over a period of 5 yr, and recaptures were obtained mainly from commercial fishermen (Taylor and Hoenig 1990). Nevertheless, no fully satisfactory explanation for the phenomenon has been found.

Discussion

Although application of the bivariate lognormal model generated clusters with less overlap than the allometric regression model, particularly for the smaller individuals, this does not in itself imply that the lognormal is the more appropriate model. The regression models were defined to predict chela height from carapace width, not vice versa. This is reasonable, since carapace width is the measure used to determine whether a crab is of legal size. Thus, if the prediction of chela height from carapace width were the underlying objective, differentiation on the basis of the allometric regression might well be appropriate. It may be argued, however, that, for classification purposes, there is no reason that one measure should be treated differently from any other and that chela height and carapace width should be regarded as a bivariate random variable, rather than dependent and independent variables, provided, of course, that the measured crab represent a random sample of the population, i.e., there was no purposive selection of any particular size(s). The bivariate model would then seem appropriate.

In the second example, the arbitrary removal of those points with y_i exactly equal to x_i is not something that one would wish to do but seems necessary if a pathological solution is to be avoided. The difficulty arises here because of the disproportionately high incidence of these points. The problem should not arise if the number of such points were consistent with the distributional assumption (normality) in relation to the disposition of the remaining points. Thus, if, after transformation to the univariate model by taking $y_i - x_i$, there were about 12 zeros instead of 623, one would anticipate a nonpathological solution. However, although seemingly more acceptable mathematically, the adding back of 12 zeros is just as arbitrary as the exclusion of all 623. Also, for the regression model, it is less clear which points should be added back. Thus, one is left with a problem that is not easily resolved. However, individuals with $y_i = x_i$ clearly are nonmolters. From the practical point of view, it would appear quite reasonable to exclude these from the data set and classify the remaining individuals, although σ_1^2 would then be overestimated. (The appropriate correction can, however, be made.) The method outlined above must then here, as well as in the first example, be regarded as more than satisfactory. It is certainly more direct than the method presented by Conan and Gundersen (1979).

The methodology here described and illustrated is appropriate when the existence of several groups can be hypothesized but one does not have available measurement of individuals whose group membership is known a priori. There are two features that, perhaps, deserve to be emphasized. First, hypotheses concerning the number of groups can be tested, including the hypothesis that the data come from a single homogenous group. Second, each individual is assigned a probability of belonging to a group; this seems particularly useful for those individuals that appear to fall between groups.

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Appendix: Development and Restructuring of the Likelihood Equations for the Second Example

As noted in the text, in the second example the $f_j(y_i)$ become

$$f_1(y_i) = (1/\sqrt{2\pi}\sigma_1)\exp[-(y_i - x_i)^2/2\sigma_1^2]$$

$$f_2(y_i) = (1/\sqrt{2\pi}\sigma_2)\exp[-(y_i - a - bx_i)^2/2\sigma_2^2]$$

$$f_3(y_i) = (1/\sqrt{2\pi}\sigma_3)\exp[-(y_i - a(b+1) - b^2x_i)^2/2\sigma_3^2].$$

It follows that

$$\frac{\partial \log L}{\partial a} = \sum_i \frac{\Sigma_j p_j \partial f_j(y_i)/\partial a}{\Sigma_j p_j f_j(y_i)}$$

and similarly for $\partial \log L/\partial b$.

Now

$$\frac{\partial f_1(y_i)}{\partial a} = 0 = \frac{\partial f_1(y_i)}{\partial b}$$

$$\frac{\partial f_2(y_i)}{\partial a} = \frac{(y_i - a - bx_i)}{\sigma_2^2} f_2(y_i)$$

$$\frac{\partial f_3(y_i)}{\partial a} = \frac{(b+1)(y_i - a(b+1) - b^2x_i)}{\sigma_3^2} f_3(y_i)$$

$$\frac{\partial f_2(y_i)}{\partial b} = \frac{(y_i - a - bx_i)x_i}{\sigma_2^2} f_2(y_i)$$

and

$$\frac{\partial f_3(y_i)}{\partial b} = \frac{(a+2bx_i)(y - a(b+1) - b^2x_i)}{\sigma_3^2} f_3(y_i).$$

As before, let

$$P(j|i) = \frac{p_j f_j(y_i)}{\Sigma_j p_j f_j(y_i)}$$

and let

$$\pi_{ji} = P(j|i)/\sigma_j^2.$$

The likelihood equations can then be written

$$\sum_i [\pi_{2i}(y_i - a - bx_i) + \pi_{3i}(b+1)(y_i - a(b+1) - b^2x_i)] = 0$$

and

$$\sum_i [\pi_{2i}(y - a - bx_i)x_i + \pi_{3i}(a+2bx_i)(y_i - a(b+1) - b^2x_i)] = 0.$$

Let $\hat{y}_i = a + bx_i$ The above equations may then be rewritten

$$\sum_i [\pi_{2i}(y_i - \hat{y}_i) + \pi_{3i}(b+1)(y_i - a - b\hat{y}_i)] = 0$$

and

$$\sum_i [\pi_{2i}x_i(y_i - \hat{y}_i) + \pi_{3i}(\hat{y}_i + bx_i)(y_i - a - b\hat{y}_i)] = 0.$$

As before, we have

$$\hat{p}_j = \sum_i \hat{P}(j|i)/n$$

and

$$\hat{\sigma}_1^2 = \sum_i (y_i - x_i)^2 \hat{P}(1|i) / \sum_i \hat{P}(1|i)$$

$$\hat{\sigma}_2^2 = \sum_i (y_i - \hat{a} - \hat{b}x_i)^2 \hat{P}(2|i) / \sum_i \hat{P}(2|i)$$

$$\hat{\sigma}_3^2 = \sum_i (y_i - \hat{a}(\hat{b}+1) - \hat{b}^2x_i)^2 \hat{P}(3|i) / \sum_i \hat{P}(3|i).$$

It remains to solve the likelihood equations for a and b. This is not as straightforward as it might seem. It must be remembered that $\pi_{ji} = \pi_{ji}(a,b)$, i.e., the π_{ji} are rather complicated functions of a and b.

The likelihood equations for a and b can be rewritten with a as subject as

$$a = \frac{\Sigma_i [\pi_{2i}(y_i - \hat{y}_i) + \pi_{3i}(b+1)(\hat{y}_i - b\hat{y}_i)]}{(b+1)\Sigma_i \pi_{3i}}$$

$$a = \frac{\Sigma_i [\pi_{2i}x_i(y_i - \hat{y}_i) + \pi_{3i}(\hat{y}_i + bx_i)(y_i - \hat{y}_i)]}{\Sigma_i \pi_{3i}(\hat{y}_i + bx_i)}.$$

An approach to solving these equations is given in the main text.

Constrained Least Squares Estimation of Mixed Population Stock Composition from mtDNA Haplotype Frequency Data

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Nuclear allozymes have provided the markers of choice for genetic stock identification, especially for Pacific salmonids. For other species, a dearth of allozyme variation has led workers to pursue mtDNA markers. Haploid mtDNA markers obviate methods based on Hardy–Weinberg equilibrium; all mtDNA markers are rigidly linked, violating the independence assumption; and mtDNA sample sizes are small. Based on a conditional weighted least squares approach for the case of known source population genotype frequencies, we develop an unconditional method for estimated genotype frequencies. We compare the methods with Monte Carlo simulation, evaluating effects of source population and mixed harvest sample sizes and those of source population divergence and mixture composition. When genetic resolution is poor or sample sizes are inadequate, the conditional method works better, but with larger sample sizes and reasonable population resolution, the unconditional method is superior.

Les allozymes nucléaires ont donné d'excellents résultats pour l'identification génétique des stocks, particulièrement en ce qui concerne les salmonidés du Pacifique. Pour d'autres espèces, la rareté de la variation des allozymes a amené les chercheurs à travailler sur les marqueurs de l'ADNmt. Les marqueurs de l'ADNmt haploïde nuisent aux méthodes basées sur l'équilibre de Hardy–Weinberg; tous les marqueurs de l'ADNmt sont liés de façon rigide, ce qui annule l'hypothèse d'indépendance; enfin, les tailles des échantillons d'ADNmt sont faibles. À partir d'une démarche conditionnelle pondérée des moindres carrés correspondant au cas où les fréquences des génotypes sont connues dans la population source, nous avons mis au point une méthode non conditionnelle pour les fréquences estimées des génotypes. Nous comparons les méthodes à la simulation Monte Carlo, en évaluant les effets de la taille de la population source et des échantillons prélevés en mélange et ceux de la divergence et de la composition mélangée de la population source. Lorsque la résolution génétique est faible, ou la taille des échantillons inadéquate, la méthode conditionnelle fonctionne mieux, mais avec des tailles d'échantillons plus grandes et une résolution raisonnable de la population, la méthode non conditionnelle donne des résultats supérieurs.

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Biological management of anadromous fish requires knowledge of proportional contributions of divergent populations to a source-ambiguous mixed harvest. Tagging studies, coupled with mark and recapture methods, have proven useful (Fraidenburg and Lincoln 1985; Johnson 1990), but much attention has also been devoted to *genetic stock identification* (GSI) (e.g., Smouse et al. 1990). The determining factor for all GSI analyses is the level of genetic differentiation among source populations (stocks). In Pacific salmonids, one can usually find about a dozen loci that are useful in any one species. In species such as Atlantic salmon (*Salmo salar*), Atlantic herring (*Clupea harengus*), striped bass (*Morone saxatilis*), and American shad (*Alosa sapidissima*), allozyme variation is too limited to be of much use for GSI, and attention has recently shifted to mtDNA markers (Bentzen et al. 1988, 1989; Davidson et al. 1989; Schweigert and Withler 1990; Wirgin et al. 1990; Nolan et al. 1991; Chapman and Brown 1992). Both nuclear allozyme and mtDNA assays are relatively cheap and easy to use, but mtDNA has two potential advantages. (1) In some species where allozymes show little variation, mtDNA is known to vary; the mitochondrion is thought to evolve faster than the nucleus. (2) The rate of mtDNA gene flow should be lower than that of nuclear genes (Birky et al. 1983; Bentzen et al. 1989), due to its uniparental inheritance, potentially enhancing source population (stock) divergence.

Haploid mtDNA markers, however, do have some unexplored statistical consequences for GSI-type analyses. Most current GSI methodology employs EM (expectation maximization) estimation (Milner et al. 1981, 1985; Beacham et al. 1985; Millar 1987; Pella and Milner 1987; Wood et al. 1987; Smouse et al. 1990), based on likelihood mixture analysis of noninterbreeding, multiple-locus Hardy–Weinberg gene pools. With strictly haploid inheritance, we sacrifice the resolution provided by departures from multiple-locus Hardy–Weinberg equilibrium (both single-locus Wahlund effect and gametic disequilibrium). Diploid inheritance is not absolutely required, of course, because we can employ

TABLE 1. Numbers of differentiable haplotypes recovered in samples from the source populations and from the mixed harvest.

Haplotype	Source populations 1	2	...	s	Harvest m
1	n_{11}	n_{12}	...	n_{1s}	n_{1m}
2	n_{21}	n_{22}	...	n_{2s}	n_{2m}
.	.	.	...	.	.
.	.	.	...	.	.
.	.	.	...	.	.
h	n_{h1}	n_{h2}	...	n_{hs}	n_{hm}
Totals	N_1	N_2	...	N_s	N_m

a type of analysis similar to that used for meristic or morphometric traits (Fournier et al. 1984), amounting to a comparison of mixed population trait frequencies with those of the potential source populations.

In return for the sacrifice of Hardy–Weinberg mathematics, we gain a large number of mtDNA haplotypes, potentially increasing resolution. All mtDNA markers are inherited as a rigid haplotype and are thus highly intercorrelated, which translates into considerable redundancy of information. Informational redundancy represents a loss of information, but computational methods have been elaborated for multiple-allelic nuclear loci (Roberts and Hiorns 1962, 1965; Bodmer and Cavalli-Sforza 1968; Elston 1971; Szathmary and Reed 1972; Thompson 1973; Long and Smouse 1983; Chakraborty and Smouse 1988; Long 1991; Chakraborty 1992), and they can be adapted to our purpose. More difficult is the observation that there are often many more haplotypes recovered than there are individuals sampled from any one stock. Different stocks share the most common haplotypes, but many individuals possess unique haplotypes seen only in a single population, or sometimes only in the mixed harvest (e.g., Bentzen et al. 1988, 1989; Davidson et al. 1989; Schweigert and Withler 1990; Wirgin et al. 1990; Nolan et al. 1991; Chapman and Brown 1992). The informational value of a collection of singletons, or how best to use it, is unclear.

Our purpose is to develop a formulation for the stock mixture problem that is designed specifically for mtDNA haplotypes. We use an extension of an iteratively reweighted (conditional) least squares procedure designed for the case where source population allele frequencies are known exactly (Pella 1986; Pella and Milner 1987). We will (a) extend the approach to the case where source population haplotype frequencies are mere estimates of the true parameters, providing an unconditional analysis, and (b) compare the newer unconditional methodology with the earlier conditional methodology by means of Monte Carlo simulation.

Statistical Methods

Our goal is to minimize the discrepancy between the observed haplotype frequency arrays of several stock and mixed harvest data sets and the frequency arrays expected under a particular mixture model. There are s source populations and h identifiable mtDNA haplotypes in the total data set. A data set consists of actual numbers of individuals, as portrayed in Table 1.

We convert the data into frequencies (y_{ij}) of the ith haplotype ($i = 1, \ldots, h$) from the jth source population ($j = 1, \ldots, s$) and harvest (y_{im}), respectively. We then define a set of observation vectors for the stocks and mixed harvest

$$\boldsymbol{Y} = \begin{bmatrix} y_{11} & y_{12} & \cdots & y_{1s} \\ y_{21} & y_{22} & \cdots & y_{2s} \\ \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots \\ y_{h1} & y_{h2} & \cdots & y_{hs} \end{bmatrix} = [\boldsymbol{Y}_1 \boldsymbol{Y}_2 \cdots \boldsymbol{Y}_s]$$

$$\text{and } \boldsymbol{Y}_m = \begin{bmatrix} y_{1m} \\ y_{2m} \\ . \\ . \\ y_{hm} \end{bmatrix}$$

which have the corresponding mixture model expectations

$$\boldsymbol{X} = \begin{bmatrix} x_{11} & x_{12} & \cdots & x_{1s} \\ x_{21} & x_{22} & \cdots & x_{2s} \\ \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots \\ x_{h1} & x_{h2} & \cdots & x_{hs} \end{bmatrix} = [\boldsymbol{X}_1 \boldsymbol{X}_2 \cdots \boldsymbol{X}_s]$$

$$\text{and } \boldsymbol{X}_m = \begin{bmatrix} x_{1m} \\ x_{2m} \\ . \\ . \\ x_{hm} \end{bmatrix}.$$

From the observed stock haplotype frequency matrix $\boldsymbol{Y}$, we need to compute the expected matrix $\boldsymbol{X}$, as well as a vector of the contributions of those stocks to the mix

$$\boldsymbol{b}' = [b_1\ b_2 \ldots b_s].$$

The expected frequency array for the mixed harvest is $\boldsymbol{X}_m = \boldsymbol{X}\boldsymbol{b}$. For nuclear loci, most algorithms are based on maximum likelihood methods. We will here employ a convenient modification of an iteratively reweighted least squares algorithm described earlier (Pella 1986; Pella and Milner 1987).

Weighted Least Squares

The object is to estimate both $\boldsymbol{b}$ and $\boldsymbol{X}$ by minimizing the sum of squared distances between observed and expected haplotype frequencies for both the stocks and the mixed harvest. The squared distance between the observations of the jth source population and their expecations is

$$\mathrm{SS}_j = N_j\,(\boldsymbol{Y}_j - \boldsymbol{X}_j)'\boldsymbol{W}_j(\boldsymbol{Y}_j - \boldsymbol{X}_j).$$

That for the mixed harvest is

$$\mathrm{SS}_m = N_m\,(\boldsymbol{Y}_m - \boldsymbol{X}\boldsymbol{b})'\boldsymbol{W}_m(\boldsymbol{Y}_m - \boldsymbol{X}\boldsymbol{b}).$$

The quantity we need to minimize is the total sum of squares

$$(1) \qquad Q(\boldsymbol{b}, \boldsymbol{X}) = \sum_{j=1}^{s} N_j(\boldsymbol{Y}_j - \boldsymbol{X}_j)'\boldsymbol{W}_j(\boldsymbol{Y}_j - \boldsymbol{X}_j) + N_m(\boldsymbol{Y}_m - \boldsymbol{X}\boldsymbol{b})'\boldsymbol{W}_m(\boldsymbol{Y}_m - \boldsymbol{X}\boldsymbol{b}).$$

The diagonal weight matrix $\boldsymbol{W}_j$ contains the multinomial weights for the jth stock, defined here as

$$(2) \qquad \boldsymbol{W}_j = \mathrm{diag}\left\{\frac{1+1/N_j}{\tilde{\boldsymbol{x}}_{ij}+1/N_j}\right\}.$$

Barring the small sample size adjustment ($1/N_j$) in both numerator and denominator, this is the weighting scheme of Pella and Milner (1987), who treated the x_{ij} as known without error. For our problem, the x_{ij} are unknown parameters, and standard estimates of x_{ij} lead to severe biases in x_{ij}^{-1} (discussion in Smouse and Chakraborty 1986), particularly where we have missing haplotypes in the sample that probably represent mere sampling zeroes. Smouse and Chakraborty (1986) showed that Eq. (2) provides a less biased and more robust choice, ensuring that all diagonal elements of $\boldsymbol{W}_j$ are nonzero, irrespective of the values of Y_{ij}. A rare or missing haplotype is weighted more heavily for a source population with a larger sample size than for one with a smaller sample size, thus avoiding the assignment of undue weight to missing haplotypes in small samples. Similarly, the weight matrix $\boldsymbol{W}_m$ is also diagonal, taking the form

$$(3) \qquad \boldsymbol{W}_m = \mathrm{diag}\left\{\frac{1+1/N_m}{\tilde{\boldsymbol{x}}_{im}+1/N_m}\right\} \text{ where } \tilde{x}_{im} = \sum_{j=1}^{s} \tilde{x}_{ij}\tilde{b}_j .$$

Barring the small sample size adjustments ($1/N_m$), these are again the Pella and Milner (1987) values, which latter are known to lead to a maximum likelihood solution for the case where the $\boldsymbol{X}_j$ are known without error. The adjusted weighting scheme yields solutions that are empirically not quite identical to the maximum likelihood solutions, but tiny departures from maximum likelihood estimates are a small price to pay for robust estimates in the presence of large numbers of sampling zeroes in the data set.

The traditional strategy is to hold the $\boldsymbol{X}_j$-vectors constant, minimizing $Q(\boldsymbol{b},\boldsymbol{X})$ with respect to $\boldsymbol{b}$, assuming that the frequency vectors are known. Differentiate $Q(\boldsymbol{b},\boldsymbol{X})$ with respect to $\boldsymbol{b}$ and solve, yielding a weighted least squares estimate

$$(4) \qquad \hat{\boldsymbol{b}} = (\boldsymbol{X}'\boldsymbol{W}_m\boldsymbol{X})^{-1}\boldsymbol{X}'\boldsymbol{W}_m\boldsymbol{Y}_m.$$

The estimate is conditioned on known $\boldsymbol{X}$ and is referred to as the conditional solution. In practice, haplotype frequencies are estimated from (often small) samples, so we will minimize $Q(\boldsymbol{b},\boldsymbol{X})$ with respect to the $\boldsymbol{X}_j$-vectors as well, obtaining *unconditional* estimates. We begin by filling the $\boldsymbol{X}$-matrix with a set of initial values, most conveniently the observed values from the $\boldsymbol{Y}$-matrix. Holding this initial $\boldsymbol{X}$-matrix constant, we solve for the traditional estimate of $\boldsymbol{b}$ in Eq. (4); that is, we begin with the conditional solution. The next step is to update each of the $\boldsymbol{X}_j$ in turn. Begin with $\boldsymbol{X}_1$, while holding $\boldsymbol{b}$ and all the other $\boldsymbol{X}_k$ ($k \neq 1$) constant; differentiate $Q(\boldsymbol{b},\boldsymbol{X})$ with respect to $\boldsymbol{X}_1$ and solve, a strategy that leads to

$$(5) \qquad \hat{\boldsymbol{X}}_1 = [N_1\boldsymbol{W}_1 + b_1^2 N_m \boldsymbol{W}_m]^{-1}\left[N_1\boldsymbol{W}_1\boldsymbol{Y}_1 + b_1 N_m \boldsymbol{W}_m\left(\boldsymbol{Y}_m - \sum_{k\neq 1}^{s}\boldsymbol{X}_k b_k\right)\right].$$

The next step is to update $\boldsymbol{X}_2$, holding $\hat{\boldsymbol{b}}$ from Eq. (4) and all $\boldsymbol{X}_k$-vectors ($k \neq 2$) constant, but replacing the original $\boldsymbol{X}_1$ with the updated estimate from Eq. (5). Now, differentiate $Q(\boldsymbol{b},\boldsymbol{X})$ with respect to $\boldsymbol{X}_2$ and solve, a strategy that leads to

$$(6) \qquad \hat{\boldsymbol{X}}_2 = [N_2\boldsymbol{W}_2 + b_2^2 N_m \boldsymbol{W}_m]^{-1}\left[N_2\boldsymbol{W}_2\boldsymbol{Y}_2 + b_2 N_m \boldsymbol{W}_m\left(\boldsymbol{Y}_m - \sum_{k\neq 2}^{s}\boldsymbol{X}_k b_k\right)\right].$$

Continue in this fashion, updating each of the $\boldsymbol{X}_j$ in turn. A second iteration begins by substituting the updated values of $\boldsymbol{X}$, $\boldsymbol{W}_m$, and the $\boldsymbol{W}_j$ into Eq. (4) and repeating the entire process, continuing until convergence.

Linear Contraints

The b_j are the fractional contributions of the various source populations to the mixed harvest, and if the mixed harvest contains individuals from those sources only, then the b_j should sum to unity and each b_j should be nonnegative. Haploid frequencies for the jth source population should also sum to unity and individual frequencies should be nonnegative. We express these constraints as

$$(7) \qquad \boldsymbol{b}'\boldsymbol{1}_s = 1 \text{ and } \boldsymbol{b} \geq 0$$

$$(8) \qquad \boldsymbol{X}_j'\boldsymbol{1}_h = 1 \text{ and } \boldsymbol{x}_j \geq 0$$

for $j = 1, 2, \ldots, s$. The iterative scheme provided by Eq. (4)–(6) is not guaranteed to satisfy the constraints in Eq. (7) and (8). In human admixture analysis, even with $\boldsymbol{X}$ taken as known, the estimated $\boldsymbol{b}$-vector frequently violates these constraints (e.g., Long and Smouse 1983; Long 1991; Chakraborty 1992). We are also concerned here with the $\boldsymbol{X}$-matrix, since it is to be estimated. We need to refine the estimates from Eq. (4)–(6).

To solve this problem, Pella (1986) and Pella and Milner (1987) have borrowed a technique called nonlinear programming from operations research that minimizes a criterion, such as Q, subject to a set of linear constraints. They assume $\boldsymbol{X}$ known, so Q reduces to a quadratic in $\boldsymbol{b}$ that can be rewritten as

$$(9) \qquad Q(\boldsymbol{b}) = K + \boldsymbol{b}'\boldsymbol{H}_b\boldsymbol{b} + \boldsymbol{c}_b'\boldsymbol{b}$$

where K is a constant that does not involve $\boldsymbol{b}$ and that will cancel from the argument and where $\boldsymbol{H}_b$ and $\boldsymbol{c}_b$ take the forms

$$(10) \qquad \boldsymbol{H}_b = N_m\boldsymbol{X}'\boldsymbol{W}_m\boldsymbol{X} \text{ and } \boldsymbol{c}_b = -2N_m\boldsymbol{X}'\boldsymbol{W}_m\boldsymbol{Y}_m.$$

They minimize $Q(\boldsymbol{b})$, subject to the constraints of Eq. (7), iterating $\boldsymbol{b}$ to a constrained solution, using the well-known Wolfe (1959) algorithm.

We will here carry that strategy one step farther, estimating $\boldsymbol{X}$ as well, subject to the constraints of Eq. (8). The function $Q(\boldsymbol{b},\boldsymbol{X})$ is now higher than second order, but we can finesse its minimization by treating Q first as a quadratic in $\boldsymbol{b}$ (with $\boldsymbol{X}$ fixed) and then as a quadratic in $\boldsymbol{X}$ (with $\boldsymbol{b}$ fixed), iterating back and forth to convergence. Our first step is to estimate $\boldsymbol{b}$, as above. We then express Q as a quadratic function of $\boldsymbol{X}_1$, holding $\boldsymbol{b}$ and all the other $\boldsymbol{X}_k$ ($k \neq 1$) constant:

$$(11) \qquad Q(\boldsymbol{X}_1) = K + \boldsymbol{X}_1'\boldsymbol{H}_1\boldsymbol{X}_1 + \boldsymbol{c}_1'\boldsymbol{X}_1$$

where K is now a constant that does not involve $\boldsymbol{X}_1$ but that will again cancel, and

(12) $H_1 = N_1 \boldsymbol{W}_1 + N_m b_1^2 \boldsymbol{W}_m$ and

$$\boldsymbol{c}_1 = -2\left[N_1 \boldsymbol{W}_1 \boldsymbol{Y}_1 + N_m b_1 \boldsymbol{W}_m \left(\boldsymbol{Y}_m - \sum_{k \neq 1}^{s} \boldsymbol{X}_k b_k \right) \right].$$

Now, we minimize $Q(\boldsymbol{X}_1)$, subject to the constraints of Eq. (8). After solving Eq. (11), we move on to the second source population, cycling back through Eq. (8), (11)–(12) with the subscript 2 replacing the 1. When all the source populations have been updated, the initial $\boldsymbol{X}$, $\boldsymbol{W}_m$, and $\boldsymbol{W}_j$ in Eq. (9) and (10) are replaced by their updated values to start another iteration on $\boldsymbol{b}$. We continue the process to convergence, having minimized $Q(\boldsymbol{b}, \boldsymbol{X})$, subject to the entire set of linear constraints. We have modified the asymptotic method of Hildreth (1957) and D'Esopo (1959) for this specific problem, denoting the final *unconditional* solutions as $\tilde{\boldsymbol{b}}$ and $\tilde{\boldsymbol{X}}_j$. We have written a small program called SHADRACQ for this purpose, available from C.J.K. on request.

Monte Carlo Simulations

Performance Criteria

We need a performance criterion for comparison of our estimators. The quadratic criterion, $Q(\tilde{\boldsymbol{b}}, \tilde{\boldsymbol{X}})$, is an obvious candidate. Unconditional estimation should never yield a larger Q-value than conditional estimation. If the observed haplotype frequencies for the stocks, $\boldsymbol{Y}$, provide optimal estimates of $\boldsymbol{X}$, given all the constraints, as sometimes happens with small data sets, then Q does not improve after the first (conditional) estimate of $\boldsymbol{b}$. More generally, Q improves with iteration. Of course, Q is neither the only nor necessarily the best performance criterion. Variance, squared bias, and mean squared error of $\tilde{\boldsymbol{b}}$ and $\tilde{\boldsymbol{X}}$ may be better criteria. Unconstrained least squares estimates are unbiased but frequently out-of-bounds. Constrained estimates are properly bounded but biased, and their sampling distributions are difficult to determine (Werner 1990). Liew (1976) showed that constrained estimates are also asymptotically unbiased, but our samples sizes are too small for asymptotic properties to be relevant.

Our situation is complicated by the fact that the stock haplotype frequencies embedded in $\boldsymbol{X}$ are also unknown. We need the variation of both estimated $\boldsymbol{b}$ and estimated $\boldsymbol{X}$; there is probably no closed form way to examine the sampling properties of these estimators. Repeated sampling (Efron 1979) may be used to approximate the sampling distribution of the estimators, but it provides information only on the variance, and bias is important here.

Inasmuch as both the conditional and unconditional constrained estimates are biased, a minimum variance solution might be less useful than a minimum mean squared error (MSE) solution. The unweighted MSE for the multivariate problem is defined (Hoerl and Kennard 1970) as the expected squared distance of the estimated $\boldsymbol{b}$-vector from the parametric vector. Baksalary and Pordzik (1990) proposed a weighted MSE, called $\boldsymbol{W}$-weighted quadratic risk of $\tilde{\boldsymbol{b}}$, which takes into account the unequal variances of the $\tilde{b}_j$ and the covariances among them. We can partition both MSE($\tilde{\boldsymbol{b}}$) and MSE($\tilde{\boldsymbol{X}}_j$) into separate variance and bias terms. It is useful to gauge the MSE($\tilde{\boldsymbol{b}}$) on a per source population basis, so we will divide by the number of source populations. It is useful to gauge the MSE($\tilde{\boldsymbol{X}}_j$) on a per source population, per haplotype basis, so we divide by the number of source populations and the number of haplotypes. We use simulation to estimate the MSE, variance, and squared bias.

Factors Influencing Performance

There are many interacting factors that influence the performance criteria. We investigate four (1) sample size for each of the source populations, (2) sample size for the mixed harvest, (3) level of genetic differentiation among source populations, and (4) heterogeneity of source population contributions. Two additional factors, upon which we shall comment in the Discussion, are (5) the number of source populations, which we have fixed at 10, and (6) the number of possible haplotypes, which we have set at 25. We need more haplotypes than source populations for identifiability.

Sample sizes of the mixed harvest and source populations

We found that mixed harvest sample sizes of less than 100 gave very poor results except when the stocks are highly divergent, and we would consider 100 to be a bare minimum sample size for the mix. Increasing sample size of the mix above 900 did very little to improve the estimates. The square root of MSE($\tilde{\boldsymbol{b}}$) is approximately linear in the logarithm of mixed sample size, N_m, within this range, so we chose $N_m = \{100, 175, 300, 500, 900\}$, values that are about equally spaced on a logarithmic scale. We used similar methods to determine the proper range and spacing of the stock sample size. Again, the square root of MSE($\tilde{\boldsymbol{b}}$) is approximately linear in the logarithm of stock sample size. We chose $N_s = \{20, 35, 60, 100, 160\}$ to cover the range of interest. We have simulated equal sample sizes, N_s, for all stocks, although that is not a requirement of the method.

Genetic divergence among source populations

Successful resolution of a mix depends critically on the level of genetic divergence among the candidate stocks, measured as some analogue of Wright's (1965) F_{st}-statistic. We find it convenient to use a mixture-analysis analogue:

(13) $$D_{st} = \frac{1}{s}\sum_{j=1}^{s} (\boldsymbol{X}_j - \boldsymbol{X}_m)' \boldsymbol{W}_m (\boldsymbol{X}_j - \boldsymbol{X}_m),$$

$$\text{with } \boldsymbol{X}_m = \frac{1}{s}\sum_{j=1}^{s} \boldsymbol{X}_j \text{ and } \boldsymbol{W}_m = \text{diag}\{x_{im}^{-1}\}.$$

We have rescaled D_{st} to range between 0 and 1. We determined that with $D_{st} < 0.05$, the enterprise is doomed; there is insufficient genetic divergence among stocks. On the other hand, $D_{st} > 0.50$ yields estimates of $\boldsymbol{b}$ with practically no error for modest sample sizes, but the results are almost obvious by inspection, and we cannot realistically expect this level of resolution very often. We chose a series of values to span this range, $D_{st} = \{0.05, 0.10, 0.15, 0.30, 0.50\}$; spacing was logarithmic.

The value of D_{st} is determined by the structure of stock haplotype frequencies. There are an infinite number of different frequency distributions ($\boldsymbol{X}$) that can generate the same D_{st}, but we chose a stylized structure for $\boldsymbol{X}$ (see Table 2), patterned on the emerging data for mtDNA in marine fish. C is the frequency of the most common haplotype, widely shared among stocks. U is the frequency of a haplotype that has modest frequency within a particular stock but that is only rarely encountered, with probability e, in other stocks. There are also haplotypes that are individually rare in all stocks, and

TABLE 2. Underlying structure of haplotype frequencies of source populations (X-matix).

Haplotype	Source populations			
	1	2	...	s
1	C	C	...	C
2	U	e	...	e
3	e	U	...	e
.	.	.	...	.
.	.	.	...	.
.	.	.	...	.
$s+1$	e	e	...	U
$s+2$	e	e	...	e
.	.	.	...	.
.	.	.	...	.
.	.	.	...	.
h	e	e	...	e

we have assigned them probability e as well. The value of e is small enough so that the presence or absence of the haplotype is unpredictable in a small stock sample. The common haplotype (with probability C in all stocks) and the rare haplotypes (each with probability e in all stocks) provide no resolution at the parametric level. Resolution is built into the difference between U and e. By setting e = 0.01, U can be chosen to determine D_{st}. Real data will never be this simple, but the point is to explore the factors important for resolution with minimal complexity.

Source population contributions

Smouse et al. (1990) showed that resolution with maximum likelihood methods is optimized when all source populations contribute equally to the mixed harvest; heterogeneity of $\boldsymbol{b}$ will reduce resolution. A convenient way to generate $\boldsymbol{b}$-heterogeneity is to define

$$(14) \quad b_j = \alpha^j / \sum_{k=1}^{s} \alpha^k \text{ for } \alpha \geq 1 \text{ and } j = 1, 2, \ldots, s.$$

As α increases, the b_j become progressively heterogeneous. The real importance of heterogeneity among the b_j is that it reduces usable resolution for a given level of D_{st}. In practice, we have usable resolution given by

$$(15) \quad M_{st} = \sum_{j=1}^{s} b_j (\boldsymbol{X}_j - \boldsymbol{X}_m)' \boldsymbol{W}_m (\boldsymbol{X}_j - \boldsymbol{X}_m), \text{ with}$$

$$\boldsymbol{X}_m = \sum_{j=1}^{s} b_j \boldsymbol{X}_j \text{ and } \boldsymbol{W}_m = \text{diag}\{x_{im}^{-1}\}.$$

Only if all sources contribute equally (α = 1) does M_{st} = D_{st}; otherwise, $M_{st} < D_{st}$. The square roots of MSE($\tilde{\boldsymbol{b}}$) and MSE($\tilde{\boldsymbol{X}}$) are roughly linear in the logarithm of M_{st}. We set α = {1.00, 1.25, 1.50, 1.75, 2.00}, yielding M_{st}-values = {0.150, 0.135, 0.110, 0.091, 0.077}.

Simulation Results

Generating random haplotypes

The parametric structure of stock haplotype frequencies ($\boldsymbol{X}$) was determined from D_{st}, using Table 2; that of the mix was obtained by setting $\boldsymbol{X}_m = \boldsymbol{X}\boldsymbol{b}$. We used a multinomial random number generator to create samples of haplotypes for each stock and the mixed harvest, based on their respective $\boldsymbol{X}$-vectors. We explored all five levels of each factor, holding the levels of other factors at their central settings. We also explored widely among other combinations of settings, but these transects through the center of the parameter space tell the essential story. For each setting, we conducted 500 random trials.

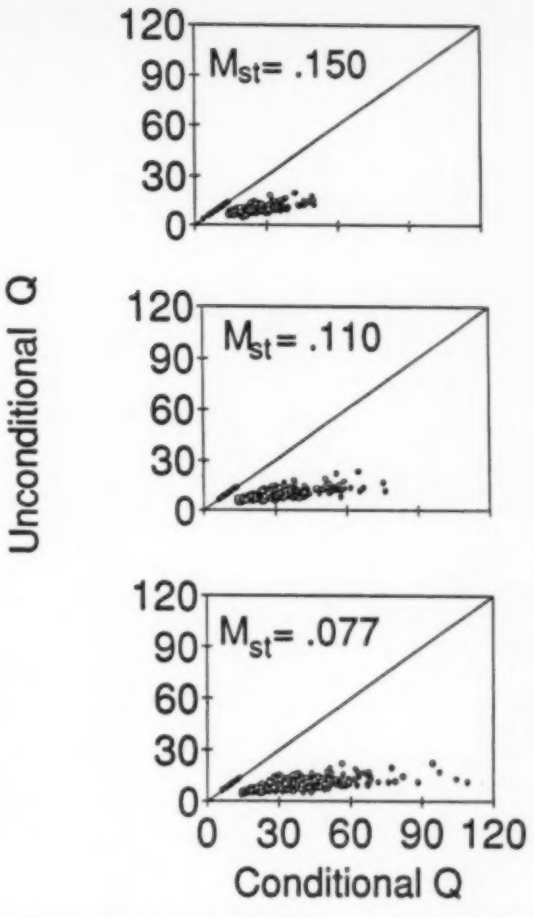

FIG. 1. Comparison of the conditional versus the unconditional Q-criterion.

The Q-criterion

To compare the Q-criterion defined above among different source sample sizes and/or different mixed sample sizes, we divided by the total sample size ($sN_s + N_m$). The following general trends were observed from the simulations: (1) Q decreases as N_s or N_m increases. (2) Increasing population divergence (D_{st}) also reduces Q, as expected. (3) Increasing heterogeneity of $\boldsymbol{b}$ (reductions in M_{st}) result in an increased Q-value for the conditional solutions, but the unconditional Q-values are relatively stable. We plotted individual Q-values in Fig. 1, unconditional values (Q_u) against conditional values (Q_c) for the top, middle, and lowest levels of M_{st}. The 45° lines represent the $Q_u = Q_c$ case, where the observed Y_j cannot be improved upon. Only when the data are inadequate do we observe points on the line, but most of the observed points fell below the line, as anticipated.

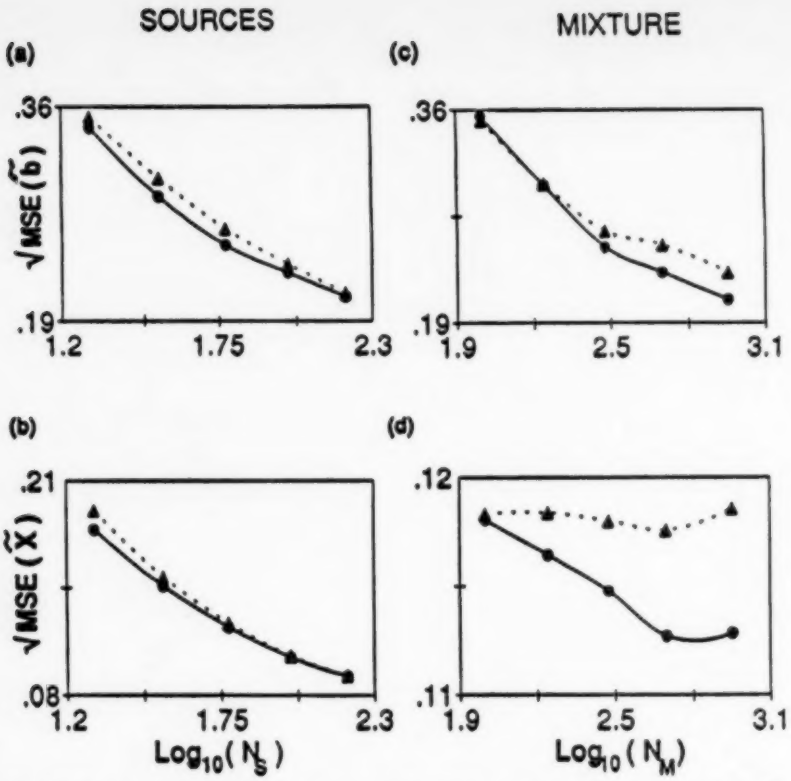

FIG. 2. Influence of sample sizes of source (N_s) and mixed (N_m) populations on MSE($\tilde{\boldsymbol{b}}$) and MSE($\tilde{\boldsymbol{X}}$).

Mean squared errors

We explored changes of MSE($\tilde{\boldsymbol{b}}$) and MSE($\tilde{\boldsymbol{X}}$) with changing levels of single factors, holding other factors constant. Figures 2a and 2b show that both MSEs decrease as the sample sizes of the stocks (N_s) increase. The unconditional estimates of $\boldsymbol{b}$ and $\boldsymbol{X}$ are generally better than their conditional counterparts, especially in the small sample size range. As N_s increases, the results of the two methods converge. The observed stock haplotype frequency arrays, $\boldsymbol{Y}$, act as a statistical anchor for the analysis, leaving very little room to maneuver by iterating on $\tilde{\boldsymbol{X}}$. Moreover, by initiating the unconditional search with the conditional solution, Eq. (4), we see empirically that for most cases, $\tilde{\boldsymbol{X}} \approx \boldsymbol{Y}$. MSE($\tilde{\boldsymbol{b}}$) decreases as the sample size of the mixed harvest (N_m) increases (Fig. 2c). When N_m is small, conditional and unconditional MSE($\boldsymbol{b}$)-values show virtually no difference, but as N_m increases, the unconditional value, MSE($\tilde{\boldsymbol{b}}$), becomes progressively smaller than its conditional counterpart, MSE($\hat{\boldsymbol{b}}$). The conditional MSE($\hat{\boldsymbol{X}}$) does not depend on N_m, of course, but the unconditional MSE($\tilde{\boldsymbol{X}}$) decreases as N_m increases (Fig. 2d). Note that Fig. 2b and 2d have different scales. When plotted on the same scale, it becomes clear that the effects of N_m on MSE($\tilde{\boldsymbol{X}}$) are negligible, relative to those of N_s.

Genetic divergence of stocks has a dramatic impact on MSE($\boldsymbol{b}$) (Fig. 3a), but the differences between conditional and unconditional values are small. When D_{st} is small (little divergence), the conditional estimate is actually better than the unconditional estimate, but when D_{st} is large enough to give any hope of resolution, the unconditional results are always better. When D_{st} is so large that assignment becomes *a priori* obvious, the difference between conditional and unconditional results is trivial. MSE($\tilde{\boldsymbol{X}}$) shows the same trends with increasing D_{st} and decreasing M_{st} as with increasing N_m (Fig. 3b and 3d), but again, a change of scale shows that MSE($\tilde{\boldsymbol{X}}$) is not very responsive to anything except N_s. Figure 3c shows that increasing heterogeneity of $\boldsymbol{b}$ will reduce the resolution (decreasing M_{st}, thus increasing the MSEs) of estimation. Interestingly, at high levels of M_{st} (homogeneous b_j), the conditional estimates are better, but the unconditional estimates become better as M_{st} decreases (heterogeneous b_j). M_{st} is the main factor influencing the efficacy of conditional and unconditional strategies, and since the respective contributions of different stocks are generally heterogeneous, unconditional estimates are generally to be preferred.

Variance and bias

Table 3 lists the summary statistics for variance, squared bias, and MSE of both $\tilde{\boldsymbol{b}}$ and $\tilde{\boldsymbol{X}}$. For the equal b_j case, with $M_{st} = D_{st}$, MSE($\tilde{\boldsymbol{b}}$) consists almost entirely of variance because none of the true b_j are near 1 or 0. Even unconstrained estimators are seldom out-of-bounds, and the constrained estimates are virtually unbiased as a consequence. As the b_j become more heterogeneous, a large fraction of the unconstrained estimates of the smaller values would be out-of-bounds; the constrained estimates are bounded, of course, but at the cost of increasing bias. The unconditional

TABLE 3. Comparison of conditional versus unconditional variance, bias, and MSE of $\tilde{b}$ and $\tilde{X}$ at the center levels of the three factors under the two extreme levels of heterogeneity of b.

Heterogeneity of b	Method	$\tilde{b}$			$\tilde{X}$		
		Variance	Bias	MSE	Variance	Bias	MSE
Even	Conditional	0.31036	0.00036	0.31072	2.62104	0.89781	3.51885
	Unconditional	0.38038	0.00038	0.38076	2.53197	0.89778	3.42976
Uneven	Conditional	2.34818	1.23882	3.58699	2.60406	0.89876	3.50282
	Unconditional	1.75468	0.61476	2.36944	2.41529	0.90716	3.32245

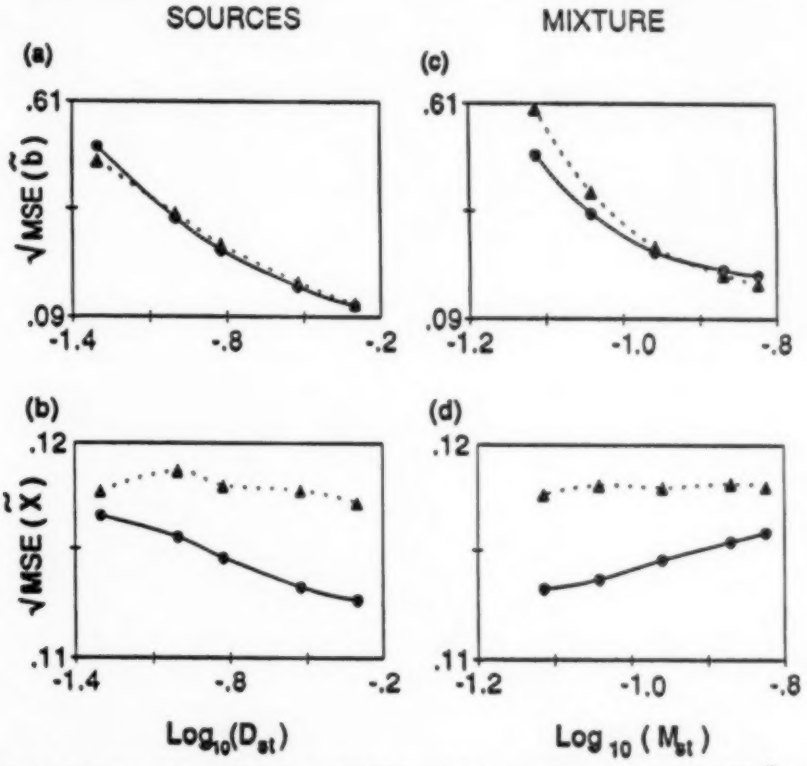

FIG. 3. Influence of population diversity (D_{st}) and heterogeneity of b (M_{st}) on MSE($\tilde{b}$) and MSE($\tilde{X}$).

MSE($\tilde{b}$) is much smaller than the conditional value, MSE($\hat{b}$), in part because of smaller variance, but mostly because of dramatic reductions in the bias. Note that each of our X_j-vectors is strongly heterogeneous, as are those of real populations, having several haplotypes of small frequency, a fact that translates into considerably biased estimates. The unconditional method improves MSE(X) at the cost of a small increase in the bias.

Uniqueness

Pella and Milner (1987) have shown that as long as b is identifiable (tantamount to the Y_j being linearly independent), any internal maximum likelihood solution (conditionally minimized Q solution) is unique. We have shown (Smouse et al. 1990) that with the unconditional algorithm, with the Y_j being treated as mere estimates of the X_j, it is possible to have more than one likelihood peak (and hence more than one Q minimum). As a practical matter, however, by initiating the iterative minimization of b and X with the conditional solution, we initiate the algorithm in the parametric neighborhood of the raw data. The net result is that the algorithm finds the solution nearest its starting point, and the two solutions are generally quite similar, although not identical. The difference is provided by the fact that there is useful information in the mixed harvest on the X_j, and sample size realities being what they are, there is sometimes more information on the jth stock from the mixed harvest than from the jth baseline sample itself. The best way to avoid uniqueness difficulties is to increase stock sample sizes; with large N_s, the conditional $X(=Y)$ acts as a statistical anchor for the unconditional analysis, and the two solutions become virtually the same.

The shifting target problem

The unconditional algorithm has one additional advantage. Stock samples are usually small and taken at a single

point in time. There is some variation over time in the genotypic arrays of the stocks themselves (Waples 1990), so quite apart from the sampling variation of the stock genotypic arrays, we are trying to estimate a "moving target". To treat a single sample of $\boldsymbol{Y}_j$ as both stationary over time and known without error is unrealistic. The unconditional method has the advantage of treating the $\boldsymbol{Y}_j$ as estimates. The best way to deal with the "shifting target" problem is to update the stock baselines periodically. Whether it is better to replace previous baselines or simply augment the current database with new samples is a matter deserving of further exploration; suffice it that the sample size benefits of pooling periodic samples from the same stock are substantial.

Discussion

Numbers of Source Populations and Haplotypes

While the number of candidate stocks was set constant at $s = 10$ for the simulations, s is clearly a variable that will influence the results. The more stocks we consider, the more difficult the mixture analysis becomes; choosing effectively among a large number of candidate stocks is simply not realistic in most circumstances. The larger D_{st}, the more stocks we can handle, so a preliminary assessment of D_{st} is a clear prerequisite for this sort of work. To guarantee statistical identifiability, we need $h \geq s$, but as a practical matter, we need $h > s$ to ensure reliable behavior of the algorithm, especially when D_{st} is small. For mtDNA restriction haplotype data, each stock may contain unique haplotypes, so the number of haplotypes may increase with the number of stocks considered. For a fixed number of stocks, the number of haplotypes is also related to the sample sizes of source and mixed populations (Chapman and Brown 1992). For example, with 20 polymorphic restriction sites, there are 2^{20} potential haplotypes, only a miniscule fraction of which will ever be seen in a finite data set. Most haplotypes are rare, and those observed are a stochastic sample from a potentially much larger pool; the presence or absence of particular variants should not be overinterpreted.

By increasing or decreasing the number of restriction enzymes used, the investigator can alter the number of differentiable haplotypes. The natural inclination has always been to add more polymorphic sites, in an effort to increase the number of differentiable haplotypes. With enough restriction sites, one could (in principle) convert most of the stock samples to nonoverlapping collections of singletons, but a mixed harvest would suffer the same fate. A collection of unique singletons in the mix conveys no useful information about mixed stock composition. Even variants represented sparsely in the database contribute little to resolution. We cannot yet provide firm recommendations on how many haplotypes to use for how many source populations, but $1.5s < h < 2.5s$ would seem to provide a reasonable range of values. The matter obviously warrants additional study.

Toward a Parsimonious Mixture Model

Inequality of the b_j is a major factor influencing resolution because M_{st} becomes progressively less than the maximum (D_{st}) as the b_j become progressively unbalanced. Detecting the contributions of small contributors is difficult, notwithstanding an upward bias in their estimated $\boldsymbol{b}$-values. There is much to be said for invoking as few stocks as possible, and removing the minor contributors will lead to only small increases in Q. A stepwise addition/removal strategy works well with the likelihood treatment of diploid nuclear markers (Smouse et al. 1990), and the same strategy should be efficacious here as well. To simplify the simulations, we assumed that stocks were genetically equidistant. In practice, of course, the genetic distances between pairs of source populations will vary considerably, resulting in a reduced value of D_{st}. For sets of genetically similar stocks, it will be almost impossible to convincingly establish their relative contributions (Hoerl and Kennard 1970; Smouse et al. 1990). On the other hand, if sets of similar stocks are genetically quite different from others, their collective contribution may be well estimated. One remedy for minimal stock resolution is to combine all the highly similar stocks into a "composite stock" and then estimate the contribution of the composite. An alternative is to estimate the separate contributions and then sum up the contributions to the "composite stock" after analysis. Again, the matter needs further study.

Multiple Mixed Populations

With a large archive of stock haplotype frequencies, we can facilitate analysis of several mixed harvests and can track changing patterns of migratory stock movements over time and space. We have described the analysis of a single mixture, but the treatment can easily be extended to multiple mixtures, each of which will have its own $\boldsymbol{b}$-vector. Consider a collection of s stocks, each stock potentially contributing to a collection of M different mixed harvests. The criterion to be minimized becomes

$$(16) \quad Q(\boldsymbol{B},\boldsymbol{X}) = \sum_{j=1}^{s} N_j (\boldsymbol{Y}_j - \boldsymbol{X}_j)' \boldsymbol{W}_j (\boldsymbol{Y}_j - \boldsymbol{X}_j) + \sum_{m=1}^{M} N_m (\boldsymbol{Y}_m - \boldsymbol{X}_m \boldsymbol{b}_m)' \boldsymbol{W}_m (\boldsymbol{Y}_m - \boldsymbol{X}_m \boldsymbol{b}_m)$$

where the symbols $\boldsymbol{b}_m$, N_m, $\boldsymbol{Y}_m$, $\boldsymbol{X}_m$, and $\boldsymbol{W}_m$ have the same meanings as before, but now indexed to the particular (mth) mixed harvest. The sequential minimization approach can be applied here as well; we simply cycle through each of the $\boldsymbol{b}_m$ in turn, holding all the $\boldsymbol{b}_n$ ($n \neq m$) constant, before beginnning the $\boldsymbol{X}_j$-updating cycle.

Other Extensions

An mtDNA restriction halplotype is defined as the combination of presence or absence of various restriction sites. Even for a modest number of restriction sites, the possible number of haplotypes may be unmanageably large (say far in excess of $h > 2.5s$). As mentioned previously, the number of rare haplotypes will increase with sample size, so resolution may not increase beyond a certain point. We probably need to develop some way of constraining the dimensionality of the problem while increasing sample size (hence, power). Our strategy here has been to treat all the haplotypes as equidistant from each other; different is simply different. For some organisms, there is useful information on stock divergence embedded within the evolutionary relationships among the different haplotypes, relationships that are evident in the patterns of restriction site sharing (Nei and Li 1979). It has been shown elsewhere that population separation (large D_{st} in the parlance of this paper) is more evident if the

fact that some haplotypes are more different than others is taken into account (Excoffier et al. 1992). We will not discuss that possibility here, except to note that a series of $g < h$ linear transforms are sufficient to effect the change, representing the desired dimensional reduction, and that g is not dependent on sample size. The $\boldsymbol{X}_j$ are replaced by vectors of "discriminant scores" that show better boundary behavior and bring some of the benefits of the Central Limit Theorem. The quadratic optimization form of the current analysis lends itself to linear transformation quite naturally; the whole problem becomes akin to multivariate normal mixture analysis.

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Concentrations of Heavy Metals and Selenium in Tissues and Some Foods of Walrus (*Odobenus rosmarus rosmarus*) from the Eastern Canadian Arctic and Sub-Arctic, and Associations between Metals, Age, and Gender

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Wagemann, R., and R.E.A. Stewart. 1994. Concentrations of heavy metals and selenium in tissues and some foods of walrus (*Odobenus rosmarus rosmarus*) from the eastern Canadian Arctic and sub-Arctic, and associations between metals, age, and gender. Can. J. Fish. Aquat. Sci. 51: 426–436.

Atlantic walrus (*Odobenus rosmarus rosmarus*) harvested at Igloolik and Hall Beach (Foxe Basin) and Inukjuak and Akulivik (eastern coast of Hudson Bay) and clams (*Mya truncata*) and cockles (*Serripes groenlandicus*) collected at Igloolik were analyzed for heavy metals and Se. Pb was high in walrus from both Hudson Bay and Foxe Basin compared with other marine mammals, but levels were higher in animals from Hudson Bay than Foxe Basin, showing a geographic trend of increasing concentration from north to south. In some tissues of clams, Pb was also high, up to 3 μg/g (wet weight). Hg levels in walrus were low compared with those in other Arctic marine mammals; in liver, Hg showed a geographic trend similar to Pb. Cd in liver and particularly in kidney of walrus was high compared with other Arctic marine mammals and was higher in those from Foxe Basin than Hudson Bay. Associations among metals and between metals and age were found in walrus that were also reported for other marine mammals, namely between Hg and Se in liver, Hg and Cd in liver, Hg and Cd with age in kidney, Cd with Zn in liver and kidney, and Hg and Se in liver with gender.

Nous avons recherché les métaux lourds et le Se chez des morses de l'Atlantique (*Odobenus rosmarus rosmarus*) prélevés à Igloolik et à Hall Beach (bassin de Foxe) et à Inukjuak et Akulivik (est de la baie d'Hudson) ainsi que chez des myes (*Mya truncata*) et des coques (*Serripes groenlandicus*) prélevées à Igloolik. La concentration de Pb était élevée chez les morses tant de la baie d'Hudson que du bassin de Foxe par rapport à d'autres mammifères marins, mais était supérieure chez les animaux de la baie d'Hudson par rapport à ceux du bassin de Foxe, ce qui montre une tendance géographique à l'accroissement de la concentration du nord vers le sud. Dans certains tissus des myes, la teneur en Pb était aussi élevée, pouvant aller jusqu'à 3 μg/g (poids humide). La teneur en Hg chez les morses était faible par rapport à d'autres mammifères marins de l'Arctique; dans le foie, on observait pour le Hg une tendance géographique similaire à celle du Pb. La teneur en Cd du foie, et particulièrement du rein, chez les morses était élevée par rapport à d'autres mammifères marins de l'Arctique, et était plus élevée chez les morses du bassin de Foxe que chez ceux de la baie d'Hudson. Nous avons observé chez les morses des associations parmi les métaux et entre les métaux et l'âge, observations qui ont aussi été signalées pour d'autres mammifères marins, notamment entre Hg et Se dans le foie, Hg et Cd dans le foie, Hg et Cd avec l'âge dans le rein, Cd avec Zn dans le foie et le rein et enfin Hg et Se dans le foie en fonction du sexe.

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In Canada the Atlantic walrus (*Odobenus rosmarus rosmarus*) occurs in northern Hudson Bay – Foxe Basin, around Baffin Island, and in the Canadian high Arctic. It is hunted wherever it occurs as food for humans and dogs. It is therefore important to know what levels of contaminants are in walrus tissues that may be consumed by humans. An earlier review of contaminants in marine mammals (Wagemann and Muir 1984) did not reveal any data on Atlantic walrus from the Canadian Arctic or northern Quebec. Although there are some data on metals for this species from Greenland and Alaska (Born et al. 1981; Taylor et al. 1989; Warburton and Seagars 1993), there appear to be no such data for this species from the Canadian North.

The main objective of this investigation was to provide background information on metals in animal tissues, which might serve as a basis for assessing possible risks to animals from toxic metals before further hydroelectric developments in the North take place. Consumers also need information on heavy metals and potential rates of intake. Other objectives were to examine geographic trends of metals in walrus, differences among marine mammal species, and elucidate relationships among metals, age, and gender.

We determined heavy metal and Se concentrations in three tissues (liver, kidney, muscle) of Atlantic walrus from Foxe Basin and eastern Hudson Bay. Walruses are primarily benthic feeders and bivalve molluscs are the major part of their

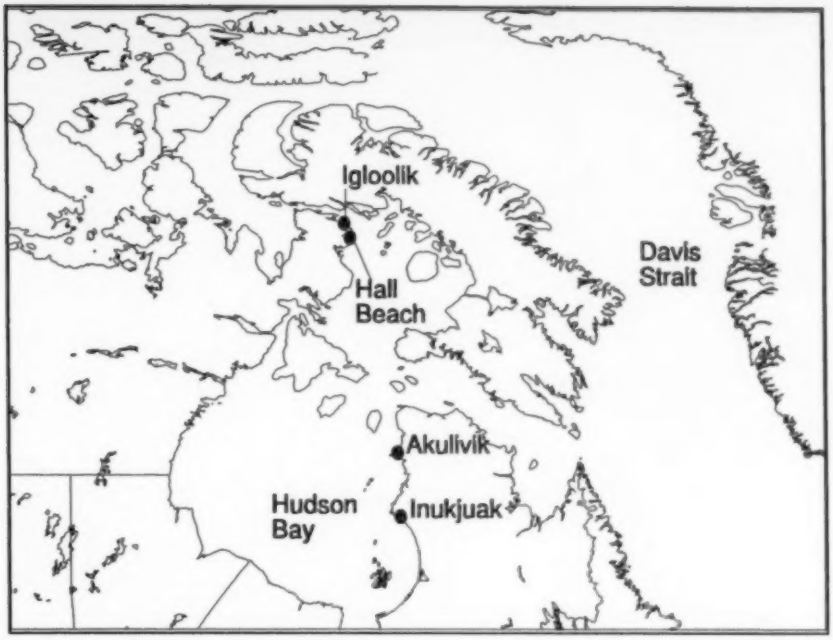

FIG. 1. Locations of sampling sites of walrus used in this study.

diet (Fisher 1989). We have therefore also measured metals in the clam (*Mya truncata*) and cockles (*Serripes groenlandicus*) to determine the primary dietary metal source for walrus.

Methods

We obtained walrus tissue samples from four communities (Table 1). Associates accompanying Inuit hunters during their subsistence hunt in the summer excised the required tissues from walrus carcases. Hunts took place at the edge of the pack ice in Foxe Basin, roughly east of the communities of Igloolik and Hall Beach, N.W.T., and in Hudson Bay at Akulivik and Inukjuak, Quebec, Canada (Fig. 1). Hunters from Hall Beach and Igloolik hunt the same stock of walrus, often within sight of each other. However, hunter selectivity for age and sex may have been different for the two communities and the samples were analyzed separately. Immediately postmortem, while the animals were being butchered, samples of about 500 g of kidney, liver, and muscle were excised with a clean knife, put into precleaned polyethylene bags, frozen in the field, and shipped on ice to the Freshwater Institute, Winnipeg, where they were stored at −30°C until analyzed. Liver was taken from animals at all four locations. Kidney and muscle were taken only from animals at Foxe Basin (Igloolik and Hall Beach). The sampling method has been described previously (Wagemann et al. 1983).

Ages of walrus were determined by counts of dentinal annuli in longitudinal thin sections of mandibular teeth (Garlich-Miller et al. 1993), assigning one growth layer group to a year (Fay 1982). To minimize variations, all sections were examined by the same observer and were read three to five times each as blind replicates (Stewart and Lavigne 1980). Statistical outliers were discarded, and the median of the remaining values was used as the final age estimate. Ages were available for most animals from each location except Igloolik from 1982 to 1984.

TABLE 1. Location and year of collection, number of animals sampled for metal analyses, mean age, SD, range, and number of walrus aged. — = data not available.

Location	Collection year	No. of animals sampled	Mean age ± SD (no. of animals aged) and range of ages
Igloolik	1982	25	—
Igloolik	1983	25	—
Igloolik	1984	31	—
Igloolik	1987	16	12.9 ± 6.6 (16) 0+ to 25
Igloolik	1988	16	8.2 ± 6.1 (10) 0+ to 16
Hall Beach	1989	17	16.9 ± 10.2 (14) 4 to 33
Akulivik	1990	4	7.3 ± 4.6 (4) 2 to 16
Inukjuak	1990	9	12.4 ± 10.4 (8) 0+ to 23

Twenty-three bivalves (12 *M. truncata* and 11 *S. groenlandicus*) were collected near Igloolik and shipped to our laboratory. The sampling site was near walrus feeding areas. External measurements (to the nearest millimetre) taken in the laboratory using callipers were shell length, height (from the umbo across the shell), and thickness. The whole weight was recorded with the clam partially thawed. Dissection

was performed as for subsampling of walrus tissues on an acid-washed Teflon tablet rinsed with triple-distilled water between dissections of individual clams. *Mya truncata* were dissected into sheath, siphon, and body; the body represented all the internal organs of the clam. *Serripes groenlandicus*, not having a sheath, were dissected into foot and body. The clam tissues were not cleaned or rinsed, since the analytical results were to represent the clam's natural state upon which walrus prey. Clam tissues were analyzed fresh without prior drying. The individual tissue components were homogenized by chopping and mixing with a stainless steel scalpel. Two portions of each tissue (0.4–1.0 g) were digested separately in quartz test tubes, one for Hg and one for the other metals (Pb, Cd, Cu, Zn), and analyzed as for walrus tissues.

Walrus tissues were first dried and ground and then analyzed for Cu, Zn, Cd, Hg, Pb, and Se according to previously described methods (Wagemann et al. 1983). Heavy metals data generated by this laboratory for various biota have been verified and discussed elsewhere (Wagemann et al. 1983; Wagemann and Armstrong 1988). Moisture content of individual samples was determined. Multiplication factors (0.299 for liver, 0.232 for kidney, 0.266 for muscle) for conversion from dry to wet weight were calculated using the average moisture content in each tissue of all animals.

The total, analytical, and interanimal coefficients of variation for the mean metal concentrations in liver, kidney, and muscle were as follows (— = data not available):

	Pb	Cd	Zn	Cu	Hg	Se
Liver						
Total	85	61	22	80	78	40
Analytical	8.7	3.8	2.3	4.3	3.6	10
Interanimal	84.6	60.9	21.9	79.9	77.9	38.7
Kidney						
Total	—	53	20	46	40	—
Analytical	—	3.5	3.7	3.6	4.5	—
Interanimal	—	52.9	19.7	45.9	39.8	—
Muscle						
Total	234	93	188	30	130	43
Analytical	17	34	1.9	1.5	8.1	5.9
Interanimal	233.4	86.6	197.99	29.96	129.8	42.6

The total coefficients of variation were derived from standard deviations for means of Igloolik samples, the analytical coefficients of variation were derived from five replicate analyses (each, including a digestion) of the same sample, and the interanimal coefficients of variation were estimated using the formula $C_i^2 = C_t^2 - C_a^2$ where C_i, C_t, and C_a are the interanimal, total, and analytical coefficients of variation. The total variation was due primarily to interanimal variation with little analytical variation for all metals and tissues.

Data for each metal and tissue from the different geographic locations were checked for normality using the Martinez and Iglewicz (1981) test. Most groups, but not all, passed the test. Multiple means were compared using Scheffé's (1959) multiple means test ($\alpha = 0.05$) which is fairly robust toward nonnormality. Means for different years were compared and then pooled at each location and compared between locations. The *t*-test ($\alpha = 0.05$) was used to compare only two means.

Robust (Andrews' sine) multiple linear regression (Andrews 1974) was performed on untransformed data (dry weight) of the largest data set (Igloolik) using all metals, age, and sex as variables. All variables were designated sequentially once as the dependent variable and once as the independent variable, and variables were retained only when they were significant as both. A critical probability level of 0.95 was chosen for retention of any variable in the model. Gender differences regarding the few variables (Hg, Se) remaining in the model were finally examined by regressions on the pooled data set from all locations.

Results

Overview

We first examined our samples from the different locations for differences in factors other than location. The age distribution of each of the groups (Igloolik, 1987 and 1988, Hall Beach, Akulivik, Inukjuak) was normal (Martinez and Iglewicz test, $\alpha = 0.05$). Multiple-means comparison (Scheffé's test, $\alpha = 0.05$) showed no significant differences in mean ages among these groups, largely because the range of ages in each group was wide. We then examined the five subgroups from Igloolik, each taken in a different year and consisting of 16–31 animals per subgroup, for temporal variations of metals in tissues and found that there were no differences among them except for Zn and Cu in muscle of the 1982 subgroup which differed from some other years. The Igloolik subgroups were then combined into one group. We then compared the four groups (Igloolik, Hall Beach, Akulivik, Inukjuak) for significant differences. Group means (Fig. 2 for Pb, Cd, and Hg in liver) were not significantly different for any of the metals in kidney and muscle, but were significantly different for some metals in liver. There were no significant differences between Igloolik and Hall Beach, and therefore, no hunter selectivity indicated. Similarly, there were no significant differences between Akulivik and Inukjuak for any of the metals. We therefore pooled Igloolik and Hall Beach into one group representing Foxe Basin (Table 2) and Akulivik and Inukjuak into one group representing northern Quebec (Table 3), yielding two groups for further analysis from geographically distinct areas. The means of these two groups did differ significantly for Pb, Hg, and Cd in liver. For the northern Quebec sample, only liver was available. We then performed a robust linear multiple regression analysis on each of these two groups (northern Quebec and Foxe Basin).

Although age was considered to be an important variable, age estimates were not available for all animals. Inclusion of age as a variable considerably reduced the size of the sample particularly when gender was also included. To see the influence of these variables on correlations among metals, we performed regression analysis on the larger of the two groups (Foxe Basin) with metals, age, and with gender as variables (24 males, 28 females), with metals and age but without gender (52 animals), with metals and gender but without age (84 males, 49 females), and with metals but without age and gender (135 animals). For the smaller, northern Quebec group (13 animals), gender was not included as a variable in regression analysis so as not to further reduce the sample size.

Age and Temporal Variations

The availability of walrus from different years (subgroups) at Igloolik permitted examination of temporal differences

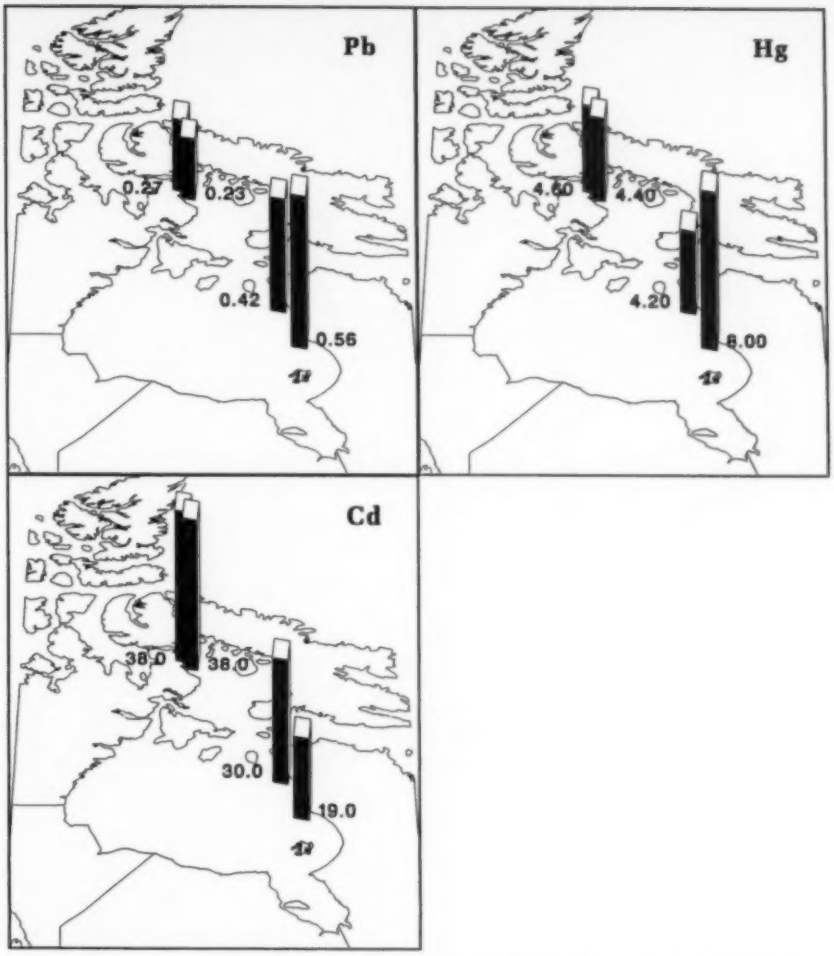

FIG. 2. Site means (μg/g dry wt), represented by the height of the bar and the numerical value at the base of each bar, of Pb, Hg, and Cd in liver of walrus from different locations in the eastern Canadian Arctic and sub-Arctic.

in metals. The annual means of Pb, Hg, and Cd (Fig. 3) in any of the tissues examined did not vary significantly (α = 0.05) from year to year. For two of the Igloolik subgroups (1987, 1988), ages were available, and the means were found not to be significantly different (α = 0.05). The relative constancy of metal concentrations reflects a stable local environmental background with respect to these metals in the vicinity of Igloolik. Mean Zn and Cu concentrations in the muscle of the 1982 subgroup were significantly higher than in other years, but this subgroup was nevertheless also pooled for the purpose of tabulation.

In the Foxe Basin group the age of the animals was positively correlated with Cd, Hg, and Se in liver (Table 4). Since tissue accumulation is governed by physiological processes, it does not contradict the finding of a constant mean concentration in groups from different years. When groups of the same mean age from different years are compared, the age factor is removed and any mean differences among metals are likely a reflection of temporal changes of the metals in the environment.

Lead

There was a significant difference in the mean Pb concentration in liver between the Foxe Basin and the northern Quebec samples, the mean being approximately twice as high in the latter (Fig. 2). There were no significant differences (α = 0.05) between locations within either of these areas themselves. Pb concentrations were not always normally distributed in each group.

Pb concentrations in the tissues of walrus did not change significantly with time during the period 1982–1988 in samples from the vicinity of Igloolik. A longer study period is needed to determine the longer term temporal trends.

Pb was comparatively high in the examined tissues of some walrus (Fig. 4a), ranging from 0.05 to 1.95 μg/g dry wt (0.02–0.58 μg/g wet wt), and in muscle from 0.01 to 1.72 μg/g dry wt (0.003–0.46 μg/g wet wt). We attribute this to the relatively high concentration of Pb in bivalves (see below), the primary food of walrus. Kidney was not analyzed for Pb. The Pb concentration in liver was on aver-

TABLE 2. Mean metal concentrations (μg/g dry wt), SD, number of samples (*n*),[a] and ranges in tissues of walrus from northern locations in Foxe Basin (Igloolik, Hall Beach combined) and collection years (1982–1988) combined. — = data not available.

	Pb	Cd	Zn	Cu	Hg	Se
			Liver			
Mean	0.26	37.6	151	32.3	4.52	9.61
SD	0.20	22.0	34.5	25.9	3.60	3.82
n	130	116	116	116	117	114
Range	0.05–1.5	0.1–137	50–300	6.2–137	0.03–19	2–20
			Kidney			
Mean	—	244	167	22.7	1.37	—
SD	—	123	33.0	10.1	0.53	—
n	—	112	112	112	112	—
Range	—	0.1–564	70–225	9.2–104	0.32–3.2	—
			Muscle			
Mean	0.078	0.52	172	3.25	0.42	12.5
SD	0.18	0.48	30.5	0.94	0.50	5.72
n	112	114	114	114	113	112
Range	0.01–1.7	0.11–1.8	85–264	11.75–7.1	0.06–5.0	1.9–28.4

[a]Indicates the number of separate samples analyzed (and not the number of replicates) and is generally the same as the number of animals collected, but can be less if each animal's organs were not all sampled or analyzed.

TABLE 3. Mean metal concentrations (μg/g dry wt), SD, number of samples (*n*), and ranges in liver of walrus from northern Quebec (Inukjuak, Akulivik) combined.

	Pb	Cd	Zn	Cu	Hg	Se
Mean	0.52	22.1	137	48.9	6.84	8.87
SD	0.47	17.6	44.9	24.0	5.48	3.42
n	13	13	13	13	13	13
Range	0.15–1.95	0.1–51	69–248	8.6–85	0.5–16	3.5–15

age approximately three times higher than in muscle. When gender was not considered, Pb in liver was correlated with Hg in both samples, and additionally with Se in the Foxe Basin sample.

Mercury

As in the case of Pb, Hg in liver of walrus varied spatially (Fig. 2). The northern Quebec sample was nearly twice as high in Hg in liver as the Foxe Basin sample. The mean Hg concentration in liver was highest in animals from Inukjuak (8.00 μg/g dry wt; 2.39 μg/g wet wt), although not significantly different from the mean at Akulivik. Similarly, there was no significant difference between Igloolik and Hall Beach.

Hg in the tissues of walrus was comparatively low (Fig. 4b). Individual concentrations ranged from 0.03 to 19 μg/g dry wt (0.007–5.68 μg/g wet wt) in liver, from 0.32 to 3.19 μg/g dry wt (0.074–0.74 μg/g wet wt) in kidney, and from 0.06 to 5.02 μg/g dry wt (0.02–1.34 μg/g wet wt) in muscle.

In the liver and kidney of the Foxe Basin sample, Hg was positively correlated with age when gender was not a variable. In the northern Quebec sample, age and Hg in the liver were also positively correlated. The regression coefficient of age on Hg was more than twice as high for the northern Quebec sample (1.91) as for the Foxe Basin sample (0.74), indicating much more rapid accumulation of Hg with age in the former sample. There was a positive correlation between Hg and Se in liver in both groups. Considering the four locations separately, the mean ratio (Hg/Se) in liver was 0.1–0.5 and depended on the mean Hg concentration in the liver at each location (Fig. 5). The relationship between the ratio (Hg/Se) and the mean Hg concentration appeared to be exponential (correlation coefficient 0.967). Notwithstanding the limited number of locations examined, the indication is that the ratio depends strongly on the mean Hg concentration.

Hg concentrations were strongly correlated between liver and kidney. The ratio of Hg in liver to that in kidney (Hg_{liver})/(Hg_{kidney}) was 2.5, indicating preferential accumulation of Hg in the liver.

Cadmium

The spatial trend for Cd was opposite to that for Pb and Hg, being significantly higher in the Foxe Basin than in the northern Quebec sample (Fig. 2). At 19 μg/g dry wt (5.7 μg/g wet wt) the mean for the Inukjuak sample was lowest among all the samples and was only half that found

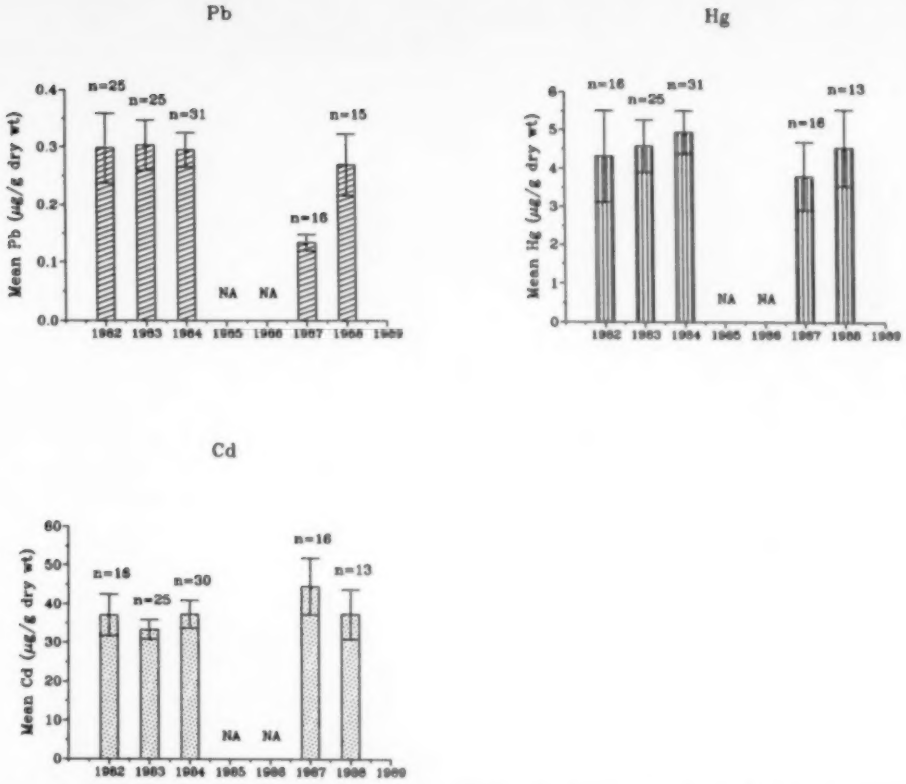

FIG. 3. Annual means ± SEM of Pb, Hg, and Cd in liver of walrus taken in different years in the vicinity of Igloolik. Each bar has a height equal to the annual group mean, and each error bar indicates 1 SEM.

in Foxe Basin and not significantly different from that at Akulivik. Similarly, the two locations in Foxe Basin (Igloolik, Hall Beach) did not differ significantly from each other.

Cd in walrus liver (Fig. 4c) was comparatively high in the Foxe Basin sample, especially in the kidney (Fig. 4d), ranging from 0.1 to 564.2 μg/g dry wt (0.03–130.9 μg/g wet wt) in kidney, from 0.1 to 137.2 μg/g dry wt (0.03–41.0 μg/g wet wt) in liver, and from 0.1 to 1.8 μg/g dry wt (0.03–0.5 μg/g wet wt) in muscle.

Cd in liver of the northern group was correlated positively with age when gender was not a variable, and only in females when gender was a variable, in both liver and kidney.

The ratio of cd in kidney to liver $(Cd_{kidney})/(Cd_{liver})$ was 6.5, indicating preferential accumulation of Cd in the kidney.

Zinc, Copper, and Selenium

Zinc and copper were measured in liver, kidney, and muscle and Se in liver and muscle. The mean concentrations of these essential elements were not expected to differ significantly among the four groups of walrus, and indeed were mostly constant and comparable with those found in other marine mammals. However, the mean Cu concentration in the liver was higher in the northern Quebec sample (14.6 ± 7.2 μg/g wet wt) than in that from Foxe Basin (9.7 ± 7.7 μg/g wet wt). More Cu was apparently available to some walrus at the southern locations. Only the two group means (arithmetic) for each metal and tissue are given (Tables 2 and 3).

Mean Se concentration did not differ significantly between the two locations, but in individual animals, they ranged (over all locations) from 2.0 to 19.5 μg/g dry wt (0.60–5.83 μg/g wet wt) in liver and were dependent on the Hg concentration. Ranges in muscle were similar to those in liver, from 1.9 to 28.4 μg/g dry wt (0.51–7.55 μg/g wet wt). Kidney was not analyzed for Se.

Cu and Zn were positively correlated in males and females, with and without age as a variable in the model. Zn and Cd in liver and kidney were positively correlated in both males and females separately and when gender was not a variable, and with and without age as a variable. This correlation was clearly very robust.

Se in liver was positively correlated with age in males and females separately (Table 4). Se and Hg in the liver were positively correlated in males and females, i.e., the higher the Hg the higher the Se concentration in males only when age was not a variable. The molar ratio (Hg/Se), 0.15, was not significantly different for male and female walrus. Se was negatively correlated with Zn in males but not in females, whether or not age was a variable in the model.

Associations

Robust multiple linear regression was performed ($P \leq 0.05$) on metals (and Se) with all combinations of age and gender as variables for the Foxe Basin sample and without gender distinction for the northern Quebec sample. In the Foxe Basin sample, when age was not included as a variable, most of the associations among metals were the same as

TABLE 4. Significant multiple linear correlations ($P < 0.05$) between metals and between metals and age with and without gender as a variable in liver, kidney, and muscle of walrus of the Foxe Basin sample. The letters L, K, and M indicate the respective tissues (liver, kidney, and muscle) and the algebraic sign associated with each letter a positive or negative correlation. If a letter does not appear in a cell, there was no significant correlation at $\alpha = 0.05$ between the two elements in that particular tissue,except for Pb and Se in kidney for which data were lacking.

	Cd	Zn	Hg	Se	Cu
			No gender distinction		
Age	L+		L+, K+		M−
Pb			L+	L+	
Cd		L+, K+		M+	
Hg				L+,	
Cu				M−	
Zn				L−	K+, M−
			Males		
Age				L+, M+	
Pb		L+	M+		
Cd		L+, K+			
Hg		K+		L+,	
Cu		L+, M−	K+		
Zn				L−	
			Females		
Age	L+, K+			L+	M−
Pb		M+	L+		L+
Cd		L+, M−	L+	M+	
Hg				M+,	
Cu		L+			
Zn				M+	

with age included. Only associations among metals with age included as a variable are shown (Table 4). Associations among metals were not always the same for all combinations of gender and age, but some associations were more robust than others and occurred with nearly all instances: Zn–Cd in kidney, in liver (except in females with age included), and in muscle of females with and without age. Correlations between Hg–Se and Hg–Pb were present in liver with most combinations of age and gender, the former being absent only in females, the latter in males when age was included, and in muscle of females when age was excluded. Neither association occurred in kidney. The association between Zn–Cu was present in liver of females and males separately with and without age, but was absent when gender was not a variable. Some additional correlations were present but they were common only to one or two combinations with age and gender and are therefore not considered further.

Four metals were associated with age: Hg, Se, Cd, and Cu. Positive correlations were Hg in both liver and kidney when gender was not a variable, Se in liver of both males and females and muscle of males, and Cd in liver when gender was not a variable and in liver and kidney of females. They signify accumulation of metals in tissues with increasing age. Cu was negatively correlated with age in the muscle of females and when gender was not a variable.

In the northern Quebec sample, multiple regression analysis of metals in liver (no other tissues were available) was performed without gender distinction. With age included in the model, age–Cd, age–Hg, Zn–Cd, Pb–Se, Hg–Se were positively correlated, as in the Foxe Basin sample, and Cu–Cd and Se–Zn were negatively correlated, also as in the Foxe Basin sample. The correlations Zn–Cd and Hg–Se were present whether or not age was in the model.

Bivalves

Morphometric data for bivalves was obtained (Table 5) but could not be used to estimate ages. The range in bivalve size spanned the average size of clam and cockle remains found in the stomachs of walrus.

Compared with levels in marine mammals the concentration of Pb in bivalve tissues was high and in the sheath of *M. truncata*, it was an order of magnitude higher than in other bivalve tissues (Table 6). Some particulate matter may have adhered to the sheath and been analyzed with the tissues. The Pb concentration ranged from 0.15 to 3.33 μg/g wet wt in the different tissues of *M. truncata*, and from 0.31 to 1.67 μg/g wet wt in *S. groenlandicus*, (Table 6).

The Hg concentrations in both *M. truncata* and *S. groenlandicus* were extremely low. Cd concentrations were also low.

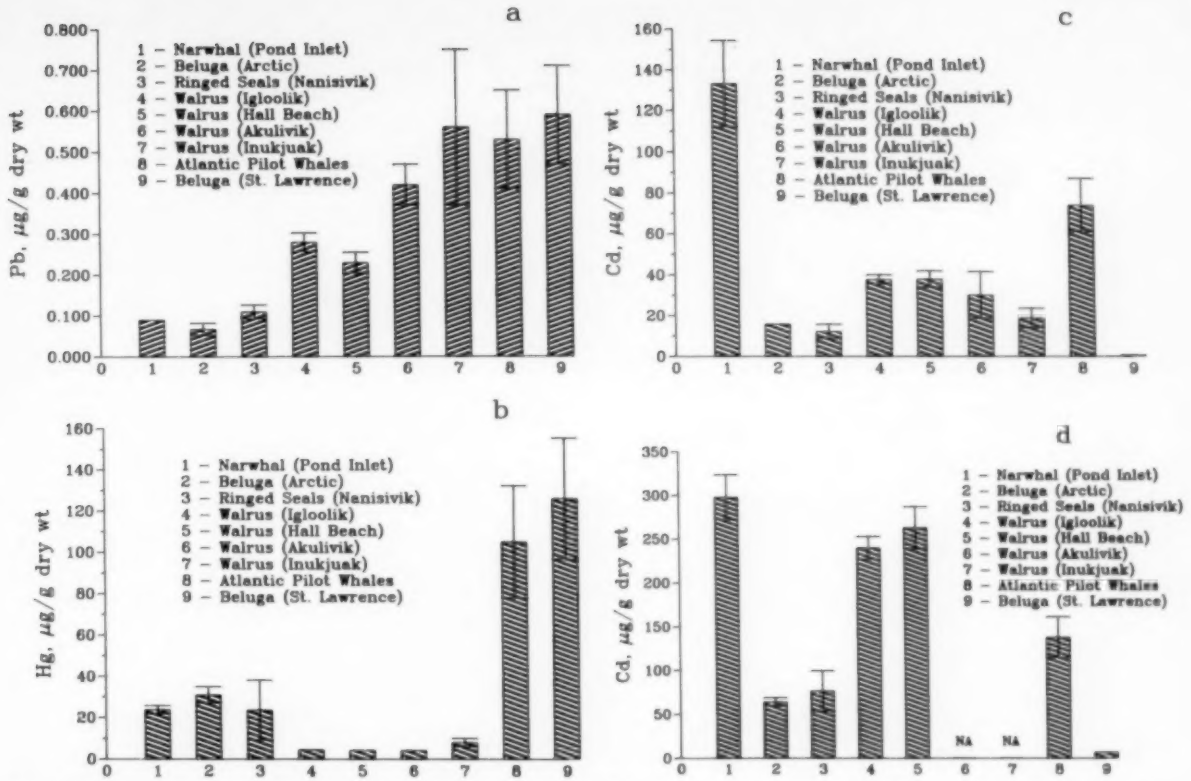

FIG. 4. Means ± SEM (a) Pb, (b) Hg, and (c) Cd in liver and (d) Cd in kidney of walrus from different locations in the eastern Canadian Arctic and sub-Arctic compared with those in similar tissues of other Arctic marine mammals. Each bar has a height equal to the group mean, and each error bar indicates 1 SEM. Data for bar graphs other than for walrus are from Wagemann et al. 1983 (1), Wagemann et al. 1990 (2, 9), Wagemann 1989 (3), and Muir et al. 1988 (8). NA, data not available.

Discussion

The data reported here for the first time for walrus from the Canadian Arctic and northern Quebec indicate the existence of geographic trends in the background levels of metals in tissues, which may be a complicating factor in any assessment of anthropogenic contaminants in the area. Pb and Hg were statistically significantly higher in animals from locations in the Hudson Bay, northern Quebec (Inukjuak, Akulivik), than in animals from Foxe Basin (Igloolik, Hall Beach), notwithstanding the relatively small sample size from the southern locations. Cd, on the other hand, was significantly higher in animals from Foxe Basin than northern Quebec.

There were no other data on metals for walrus from the Canadian Arctic with which to compare our findings. Pb in liver of walrus was twice as high (on average) in southern animals, 0.52 μg/g dry wt (0.16 μg/g wet wt), than in northern animals, 0.26 μg/g dry wt (0.08 μg/g wet wt), and was higher at all locations (Fig. 4a), in comparison with levels in Arctic beluga (*Delphinapterus leucas*), narwhal (*Monodon monoceros*), and ringed seals (*Phoca hispida*), (Wagemann 1989; Wagemann et al. 1983, 1990). In walrus from Inukjuak, Pb was as high as in beluga from the St. Lawrence and nearly as high as in white-beaked dolphins (*Lagenorhychus albirostris*) from the eastern Canadian and U.S. seaboards that had some of the highest recorded Pb values in marine mammals (Muir et al. 1988). Clams and cockles may have been the major source of Pb for walrus, judging by the correspondence between the high levels in both bivalves and walrus.

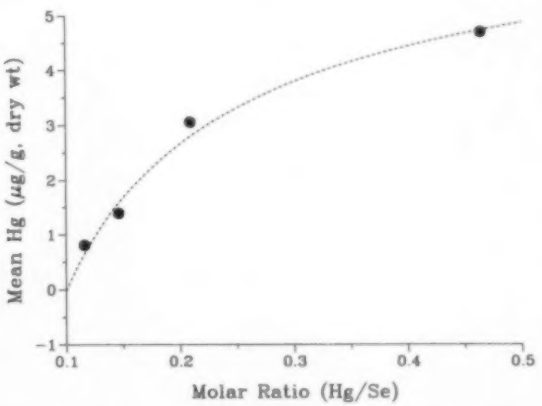

FIG. 5. Mean Hg concentration in liver of walrus from the different sampling locations in Foxe Basin and northern Quebec versus the corresponding mean ratio (Hg/Se).

TABLE 5. Average morphometric data of clams and cockles.

	Length (mm)	Height (mm)	Thickness (mm)	Whole-clam tissue wt (g)
Mya truncata	43.8 ± 7.8 *n* = 12	30.1 ± 5.0 *n* = 12	20.4 ± 3.6 *n* = 12	17.3 ± 7.9 *n* = 12
Serripes groenlandicus	56.0 ± 10.6 *n* = 6	44.0 ± 7.3 *n* = 6	28.2 ± 5.1 *n* = 6	27.6 ± 12.4 *n* = 6

TABLE 6. Average trace metal concentrations (μg/g wet wt) in different clam and cockle tissues from the vicinity of Igloolik.

Tissue	Pb	Cd	Hg	Cu	Zn
			Mya truncata		
Siphon	0.15 ± 0.08 *n* = 8	0.21 ± 0.13 *n* = 12	0.007 ± 0.005 *n* = 7	0.7 ± 0.4 *n* = 12	9.0 ± 3.2 *n* = 12
Sheath	3.33 ± 1.75 *n* = 11	0.16 ± 0.12 *n* = 11	≤ 0.005 *n* = 7	6.8 ± 3.7 *n* = 11	15.8 ± 4.2 *n* = 11
Innards	0.23 ± 0.26 *n* = 11	0.34 ± 0.17 *n* = 11	≤ 0.005 *n* = 7	2.1 ± 0.6 *n* = 11	16.7 ± 3.8 *n* = 11
			Serripes groenlandicus		
Innards	1.67 ± 0.79 *n* = 6	0.14 ± 0.04 *n* = 6	0.021 ± 0.001 *n* = 3	2.6 ± 0.8 *n* = 6	21.8 ± 8.7 *n* = 6
Foot	0.31 ± 0.06 *n* = 6	0.03 ± 0.03 *n* = 6	0.020 ± 0.004 *n* = 3	0.5 ± 0.1 *n* = 6	15.8 ± 2.5 *n* = 6

In environments where metals are readily available to them, molluscs can accumulate high levels of heavy metals and could be a major source of metals for walrus. Fallis (1982) reported Pb concentrations in clams from Strathcona Sound near the Nanisivik mine. They were strongly dependent on the proximity of the sampling locations to the ore loading terminal and ranged in total soft tissue of *M. truncata* from 6.96 to 0.19 μg/g dry wt (0.81–0.02 μg/g wet wt, using a conversion factor of 0.116). In *S. groenlandicus*, they ranged from 4.00 to 1.54 μg/g dry wt (0.92–0.36 μg/g wet wt, using a conversion factor of 0.231) (Fallis 1982). Although we did not analyze homogenates of total soft tissue, these ranges are comparable with those we found in specific tissues.

Because bivalves were collected only near Igloolik, a broader study, focusing on contaminants in bivalves and encompassing more sampling sites, will be needed to determine the spatial distribution of metals in these organisms in Hudson Bay coastal waters and to better understand the sources of metals to walrus and their geographic distribution.

The geographic trend in the level of Cd in tissues of walrus, higher in animals from Foxe Basin than from northern Quebec, was opposite that of Hg and Pb. Other marine mammals were reported to show a similar geographic trend for Cd. For example, Cd was very low in beluga from the St. Lawrence, in both liver (Fig. 4c) and kidney (Fig. 4d), much higher in beluga from the Arctic (Wagemann et al. 1990), and very much higher in narwhal from the Pond Inlet area, Baffin Island, N.W.T. (Wagemann et al. 1983). Similarly, in comparison with other belugas (Hansen et al. 1990; Wagemann et al. 1990) and ringed seals (Wagemann 1989), Cd was relatively high in walrus liver (Fig. 4c). It was even higher in the kidney (Fig. 4d), exceeded only by the high levels found in narwhal organs (Wagemann et al. 1983). However, clam tissues were relatively low in Cd (Table 6), and therefore, clams may not have been the primary source of Cd for walrus. Instead, walrus may derive some Cd directly from sediments ingested with food.

Marine mammal tissues, including walrus tissues (liver and flesh), are a staple in the Inuit diet (Smith and Armstrong 1975; Kinloch et al. 1992). Toxic metals in such animals are therefore a concern to consumers. The Canadian Federal Guideline for Hg in fish (0.5 ppm, or mg/kg wet wt), provides for a safe consumption rate of 0.31 kg fish/wk (Minister of National Health and Welfare 1984) and was promulgated specifically for Hg in fish and does not necessarily apply also to marine mammals. In Arctic and northern Quebec coastal communities where consumption rates of fish are generally higher than in southern regions, the federal guideline for total Hg in fish is 0.2 ppm (or mg/kg wet wt) (Minister of National Health and Welfare 1984).

Hg levels in tissues of walrus from all locations were lower than in other marine mammals (pilot whales (*Globicephala malaena*), Muir et al. 1988; ringed seals, Wagemann 1989; beluga, Wagemann et al. 1990) and much lower than in beluga from the St. Lawrence (Fig. 4b). In muscle of walrus, none of the Hg means exceeded the federal guideline of 0.5 ppm (or mg/kg wet wt), nor the lower one (0.2 ppm, or mg/kg wet wt) for fish; the overall mean for all groups combined was 0.11 μg/g wet wt. In liver mercury was higher (1.42 μg/g wet wt, all groups combined) and did exceed the guideline for fish, but was nevertheless comparatively low (Fig. 4b). The low Hg concentration in walrus tissues likely reflects low Hg levels in bivalve tissues (Table 6).

There are no federal guidelines for Cd and Pb in fish or marine mammals similar to that for Hg in fish. Consumer

protection in regard to these metals is based on a case-by-case assessment of potential exposure of consumers to levels of these metals (C. Charbonneau, Chemical Evaluation Division, Bureau of Chemical Safety, 4th Floor East, Banting Building, Ottawa, Ont., personal communication). For example, in an assessment of the existing Cd data for narwhal (Wagemann et al. 1983) by the Federal Department of Health and Welfare, Canada, Health Protection Branch (May 6, 1993), it was recommended that no more than 640 g of liver and 345 g of kidney be consumed per year (communication to the Inuit Regional Corporation, Inuvik, N.W.T., May 6, 1993, by H.B.S. Conacher, A/Director).

Trace metals, including toxic metals, occur naturally at low levels in the environment and many are essential for life. However, some toxic metals have increased in the marine biosphere significantly over human history (Boutron and Delmas 1980; Drasch 1983; Bourgoin and Risk 1987). This has occurred especially near polluted coastal, industrialized areas. Atmospheric loadings have also been reported offshore over the Atlantic Ocean (Slemr and Langer 1992). Marine mammals in some coastal areas are exposed to various contaminants as a result of human activities (Braham 1973; Harms et al. 1978; Viale 1978; Duinker et al. 1979; Hyvärinen and Sipilä 1984). Also in seemingly pristine areas (the Canadian Arctic and Greenland), reports indicate that Pb, Hg, and Cd are relatively high in some marine mammals (Smith and Armstrong 1975; Wagemann et al. 1983; Wagemann 1989; Hansen et al. 1990). However, specific nutritional sources of Cd and Hg in narwhal and pinnipeds in the Arctic have not been discovered to date, except for the relatively high levels of Cd found in the amphipod *Parathemisto libellula*, a prey organism of marine mammals, and especially favoured by ringed seals (Macdonald 1986).

The assessment of the toxicity by metals to marine mammals requires consideration of the antagonistic and synergistic influences by other metals and the speciation of metals in tissues. With the exception of methyl mercury, species of other metals that may influence toxicity of the total metal burden in tissues have not been determined routinely. The total Cd concentration in narwhal, for example, was reported to be sufficiently high (Wagemann et al. 1983) to expect kidney dysfunction. Although kidney tissue was not examined histologically for metal damage in that study, the animals were obviously alive and swimming when harvested. As an indicator of toxicity, the total concentration of a metal by itself, although a useful and necessary datum, is insufficient. In the case of Cd, the determination of metallothionein in tissues would appear to be a necessary adjunct to the measurement of total Cd for a better indication of its toxicity because much of the Cd in tissues is associated with this protein, a much less toxic form of Cd. The toxicity of other metals may be similarly influenced by the presence or absence of specific forms or species of the metal, such as the mitigating influence of Se on Hg. These influences sometimes manifest themselves as correlations in statistical analyses.

Some associations among metals and among metals and age (or length) appear to be common to different marine mammals generally. For example, the associations between Hg and Se in liver, between age and Hg and Cd, between Se and Pb, between Cu and Zn, and between Cd and Zn in liver and kidney of walrus (Table 4) were reported also for other marine mammals (Koeman et al. 1973; Wagemann et al. 1983, 1989, 1990; Muir et al. 1988). The mechanisms underlying these associations are largely unknown. We speculate that the associations between Cd and Zn and between Zn and Cu are probably mediated by metallothionein because these metals are inductors and constituents of this metalloprotein (Suzuki 1982). We have no explanation for the association between Pb and Se and between Pb and Hg. On the other hand, the commonly found association between Hg and Se in liver probably arises through the formation of inorganic polymorphous concretions of mercuric selenide (HgSe) (Martoja and Berry 1980; Pelletier 1985), which may also explain the protection afforded by Se against Hg toxicity and the molar ratio of 1 between Hg and Se (Hg/Se) found frequently in different marine mammals (Koeman et al. 1973). However, Hg/Se < 1 (Wagemann et al. 1983) and Hg/Se > 1 (Hansen et al. 1990) have also been reported.

The low molar ratio (Hg/Se) found in walrus liver may be related to the relatively low Hg concentration in these animals and the essential and nonessential nature of Se and Hg, respectively. Because Hg is not an essential element, it is not maintained at any particular level by homeostasis and therefore can be present at very low or high concentrations in the liver. Se, because it is an essential element, must be present at least at some minimum level to maintain good health. This minimum level is not known for marine mammals, but it may exceed the level of Hg when the latter is present at a low concentration and for which no minimum requirement is imposed by the organism. This situation may lead to a low molar ratio (Hg/Se). On the other hand, at high Hg and Se concentrations in the liver, only a small fraction of the total Se may be needed biochemically for the proper maintenance of health, and the rest would presumably be available for HgSe formation. This situation may lead to molar ratios ≥1. The high concentration of total Se in the liver of marine mammals invariably found in conjunction with high concentrations of total Hg in the liver may arise because of a continuous transformation of biochemically active Se in the liver into the relatively inert form of HgSe, the deposition of this form of Se in the liver as particles (Martoja and Berry 1980), and the consequent continuous replenishment of biochemically active Se by the organism to maintain good health.

The Hg concentration in the flesh of walrus was lower than in some other Arctic marine mammals and in muscle was much below the federal guideline for total Hg in fish (0.5 ppm, or mg/kg wet wt). Even the lower federal guideline (0.2 ppm, or mg/kg wet wt) was not exceeded by the Hg levels in the flesh of walrus we examined. In the liver and kidney, Hg exceeded the consumer guideline for fish. Pb and Cd were high in comparison with other marine mammals, and Cd greatly exceeded the consumer guideline (for Hg in fish). The health of consumers of marine mammal tissues is affected not only by the concentration of the toxic metals in the consumed tissues, but also by the frequency and quantity of foods consumed, and a lower consumption rate of the tissue then becomes the governing factor in the protection of consumers. Possible effects from sublethal levels of metals on neurological and immunological functions and behaviour of marine mammals themselves have not been studied, and the health implications for the animals are unknown.

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Heat and Cold Tolerance of the Fathead Minnow, *Pimephales promelas*, Exposed to the Synthetic Pyrethroid Cyfluthrin

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Heath, S., W.A. Bennett, J. Kennedy, and T.L. Beitinger. 1994. Heat and cold tolerance of the fathead minnow, *Pimephales promelas*, exposed to the synthetic pyrethroid cyfluthrin. Can. J. Fish. Aquat. Sci. 51: 437–440.

Critical thermal maximum (CTMax) and minimum (CTMin) techniques were used to bioassay the effects of sublethal exposures of a synthetic pyrethroid, cyfluthrin, to 240 larval fathead minnows, *Pimephales promelas*. Fathead minnows were exposed for 24 h to nominal concentrations of cyfluthrin with acetone as a carrier equal to approximately 20 and 70% of the 96-h LC50. Exposure to cyfluthrin caused maximum decreases of 3.3°C below median heat tolerance of controls (34.4°C) and a 5.6°C increase in median cold tolerance above controls (5.9°C). At the highest concentrations of cyfluthrin tested, the zone of temperature tolerance was reduced by 30%. Concentrations of cyfluthrin that caused these effects were as low as 170 parts per trillion. We believe that this is the first use of CTMin as a bioassay. Although the physical and chemical properties of cyfluthrin may preclude its presence in the environment in large amounts, extremely small concentrations can compromise the ability of fathead minnows to resist extreme temperatures. Cyfluthrin use may have greater adverse effects on nontarget species in northern climates or during colder seasons in southern climates.

La technique des températures critiques maximale (CTMax) et minimale (CTMin) a été utilisée pour rechercher les effets biologiques d'expositions sublétales à un pyréthroïde synthétique, la cyfluthrine, de 240 larves de tête-de-boule, *Pimephales promelas*. Ces poissons ont été exposés pendant 24 h à des concentrations nominales de cyfluthrine dans l'acétone égales à environ 20 et 70 % de la CL50 96 h. L'exposition à la cyfluthrine a causé une baisse maximale de 3,3 °C au-dessous de la température médiane de tolérance à la chaleur des témoins (34,4 °C) et une hausse de 5,6 °C au-dessus de la température médiane de tolérance au froid chez les témoins (5,9 °C). Pour la concentration de cyfluthrine la plus élevée des essais, la zone de tolérance à la température a été réduite de 30 %. Les concentrations de cyfluthrine qui causent des effets étaient d'à peine 170 parties par billion. Il s'agit, semble-t-il, de la première fois que la CTMin est utilisée comme épreuve biologique. En raison de ses propriétés physiques et chimiques, on peut exclure la présence en grandes quantités de la cyfluthrine dans l'environnement, pourtant des concentrations extrêmement faibles peuvent empêcher les têtes-de-boule de résister à des températures extrêmes. L'utilisation de la cyfluthrine peut avoir des effets nocifs plus importants sur des espèces non visées dans les climats nordiques ou pendant les saisons froides dans les climats méridionaux.

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Modern pyrethroids, synthetic structural derivations of the natural pyrethrins found in the flowers of *Chrysanthemum cinerariaefolium*, are among the most toxic insecticides known. For example, LD50s range from 4.64 μg phenothrin/g to 0.016 μg deltamethrin/g in tobacco budworm, *Heliothis virescens* (Miller and Salgado 1985). Although natural pyrethrins have been used as insecticides since the middle of the nineteenth century, low photostability and high biodegradability limit their effectiveness. About 1950, the first synthetic pyrethroid, allethrin, was introduced (Smith and Stratton 1986; Haya 1989); however, allethrin and other early formulations were not as efficacious against insects as natural pyrethrins. The next generation of pyrethroids, fenvalerate, permethrin, cypermethrin, and deltamethrin, were more toxic, photostable, and less susceptible to chemical and biological degradation than natural pyrethrins (Davies 1985). Most pyrethroids are lipophilic, halogenated, and have extremely low (1–100 μg/L) water solubilities (Coats et al. 1989). They strongly absorb to plants and sediments but apparently penetrate only the upper few centimetres of sediments (Solomon et al. 1985). Pyrethroids are affected by volatilization, biodegradation, and hydrolysis (Rawn et al. 1982; Maguire et al. 1989) and hence, are not highly persistent in the environment. Half-lives in soil and water range from 1 to 4 wk and from hours to days, respectively.

Since pyrethroids have no known mutagenic, carcinogenic, or teratogenic effects in mammals (Litchfield 1985) and are not highly toxic to mammals or birds, they have been utilized for household, agricultural, and veterinarian purposes (Smith and Stratton 1986; Haya 1989). Although targeted to aquatic and terrestrial insects, nontarget aquatic organisms may be adversely affected by direct application of pyrethroids or through spray drift and runoff from surrounding areas. According to Hill (1985) and Bradbury and

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TABLE 1. Five-number summaries (minimum, 25th percentile, median, 75th percentile, and maximum) for fathead minnows exposed to increasing (CTMax trials) and decreasing (CTMin trials) temperatures.

Group	*n*	Minimum	25%	Median	75%	Maximum
			CTMax, °C			
Control	30	31.4	33.8	34.4	35.0	35.9
Acetone control	30	32.3	33.2	33.9	34.6	35.8
LC10 (0.22 μg/L)	30	31.0	32.0	32.3	32.7	33.2
LC38 (0.81 μg/L)	30	30.0	30.5	31.1	31.6	32.1
			CTMin, °C			
Control	30	3.4	5.4	5.9	7.4	9.9
Acetone control	30	3.6	4.8	5.9	6.8	8.9
LC08 (0.17 μg/L)	30	7.4	9.6	11.5	12.2	13.9
LC51 (1.11 μg/L)	30	7.6	9.3	11.1	12.3	12.7

Coats (1989), fish are one to five orders of magnitude more sensitive than mammals.

The pyrethroid of interest in our study was cyfluthrin. Although synthesized in 1977, little information has been published concerning cyfluthrin; however, in his review, Leahey (1985) cited it among the most toxic pyrethroids. Cyfluthrin was ranked by Wadleigh et al. (1991) as the second most toxic of 10 different pyrethroids to german cockroach, *Blattella germanica*. The objective of this study was to quantify the effect of sublethal concentrations of cyfluthrin on the ability of larval fathead minnows, *Pimephales promelas*, to resist both increases and decreases in temperature, i.e., use critical thermal maximum (CTMax) and minimum (CTMin) to bioassay sublethal concentrations of this pyrethroid. The endpoints of these trials, measures of upper and lower temperature tolerance, are biologically important and defensible (Beitinger and McCauley 1990). Null hypotheses under test were that exposures to sublethal concentrations of cyfluthrin will not influence either the upper or lower temperature tolerance of larval fathead minnows.

Methods

Fathead minnows were obtained from a laboratory culture at the University of North Texas. Dechlorinated City of Denton tapwater was used as holding, exposure, and test water. At the time of collection, fish were less than 48 h old. Prior to temperature tolerance trials, a static 96-h LC50 was conducted to determine sublethal exposures for temperature tolerance bioassays following USEPA (1975) recommendations. Probit analysis on measured cyfluthrin concentrations yielded a 96-h LC50 of 1.08 μg/L with fiducial limits of 0.78 and 1.49 μg/L at 23°C and a pH of 8.0. Due to cyfluthrin's extremely low water solubility (0.002 μg/mL at 20°C), acetone was used as a carrier solvent.

Experiments were conducted to assess the effects of sublethal concentrations of cyfluthrin on temperature tolerance. Temperature tolerance was evaluated via critical thermal methodology as both the CTMax and CTMin. This approach was introduced by Cowles and Bogert (1944), defined by Cox (1974), and reviewed for fishes by Becker and Genoway (1979), Paladino et al. (1980), and Beitinger and McCauley (1990).

Two-day-old fathead minnows were randomly allocated to four tanks and held in water at pH 8.0 and 22–23°C. Nominal concentrations equivalent to 20 and 70% of the 96-h LC50 of cyfluthrin in an acetone carrier were added to two of the tanks. The actual measured concentrations were 0.11 and 1.11 μg/L, which are equivalent to LC08 and LC51, respectively. The two remaining tanks served as a control and acetone control. After 24 h, fish were placed two to a beaker in five 50-mL beakers with 40 mL of fresh water, positioned in a freezer capable of generating a temperature of -20°C, and exposed to a temperature decrease of approximately 0.3°C/min. CTMins were determined in three repetitions of 10 fish each. The selected CTMin endpoint was the temperature at which a fish no longer responded to gentle prodding with a glass rod.

During CTMax trials, temperature was increased from 23°C at a rate of 0.3°C/min via a Haake E-52 thermoregulator. Again, 30 individuals (three replications of 10 fish each) were tested following 24-h exposures to control, acetone control, or nominal concentrations of approximately 20 and 70% of the 96-h LC50 of cyfluthrin. Measured cyfluthrin concentrations equalled 0.22 and 0.81 μg/L, which are equivalent to 96-h LC10 and LC38, respectively. The endpoint for CTMax trials was final loss of equilibrium.

In both CTMin and CTMax trials, temperatures were monitored with a Digitec HT-5810 digital thermometer (±0.01°C) calibrated with an ASTM mercury thermometer. When a fish met the specific endpoint, it was returned to 23°C and survival was examined. Cyfluthrin concentrations were quantified from 1000-mL samples of water taken 30 min after dosing each test tank. Cyfluthrin was extracted with 5 mL of Optima hexane for 90 min and maintained at −20°C in the dark prior to analysis via gas chromatography. Samples were quantified with a Hewlett-Packard 5890 gas chromatograph with a nickel 63 electron capture detector and a 3392A integrator controlled by a Turbochrom 3 computer program. Quality assurance procedures were followed. Previous research (Hambleton 1992) had established a valid cyfluthrin detection limit of 10 ng/L.

Temperature tolerance data initially were tested for normality (Shapiro–Wilk w statistic) and inequality of variances (Hartley's F_{max} variance ratio test). Two of the eight distributions departed significantly from normal ($\alpha < 0.05$) and the CTMax data were significantly heteroscedastic ($0.05 > p > 0.01$). Consequently, data for each of the eight groups were presented as five-number summaries (minimum,

25th percentile, median, 75th percentile, and maximum) and the four CTMax groups and four CTMin groups were separately analyzed for differences via a Kruskal–Wallis nonparametric multisample test and a Student–Newman–Keuls (SNK) multiple range test on ranked temperature tolerance data. All statistical decisions were based on an α of 0.05.

Results

Control fathead minnows had median CTMax and CTMin of 34.4 and 5.9°C (Table 1). CTMaxs of fathead minnows exposed to sublethal cyfluthrin concentrations for 24 h were significantly different (Kruskal–Wallis one-way ANOVA, $p = 0.0001$). The four exposure groups were separated by their CTMaxs into three statistically distinct classes: control = acetone control > LC10 > LC38 (SNK multiple range test on ranked data, $\alpha = 0.05$). Median CTMaxs for these four groups equalled 34.4, 33.9, 32.3, and 31.1°C, respectively.

Cyfluthrin effects were even more dramatic in fathead minnows exposed to decreasing temperatures. CTMins of fathead minnows in the four exposure groups were significantly different (Kruskal–Wallis one-way ANOVA, $p = 0.0001$). An SNK multiple range test separated the four groups into two statistically distinct categories: control = acetone control > LC08 = LC51 (SNK on ranked data, $\alpha = 0.05$). Median CTMins for fathead minnows exposed to cyfluthrin, 11.5 and 11.1°C, were approximately twice the medians, 5.9°C, of the control and acetone control fathead minnows.

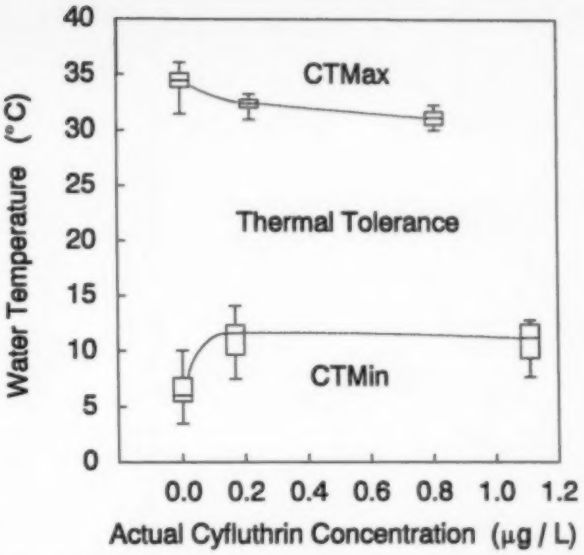

FIG. 1. Temperature tolerance ability of fathead minnows. Each boxplot, illustrating the five-number summary, is based on a sample of 30 fish. Data for the acetone control group are not shown, since they would overlap the control data.

Discussion

The median CTMax for control fathead minnows, 34.4°C, is consistent with previously published values. Watenpaugh and Beitinger (1985) and Carrier and Beitinger (1988) reported mean CTMax values for adult fathead minnows of 35.1 and 34.8°C, respectively. Pretest holding conditions of these three studies were similar. Comparing means and medians, only a 0.7°C difference exists between the CTMax values of adult and larval fathead minnows. We found no information for fathead minnows to compare with our control CTMin median of 5.9°C.

In both CTMax and CTMin tests, fish in the acetone-exposed and control groups did not differ in their ability to tolerate increasing or decreasing temperatures. Hence, acetone, at least at the concentration used in this research, 0.76 µg/L, did not affect either upper or lower temperature resistance in fathead minnows. Other chemicals that have no effect on CTMax in fishes include tetracycline (Schubauer et al. 1980) and endothal (Takle et al. 1983).

In contrast, the synthetic pyrethroid cyfluthrin significantly affected the ability of larval fathead minnows to resist changes in temperature. In CTMax trials, the median temperature for fathead minnows exposed for only 24 h to a concentration of cyfluthrin that would be expected to kill 10% of the fish in 96 h (LC10) was approximately 2.1°C less than controls. A decrease of 3.3°C from controls was measured in fish exposed to a LC38 concentration of cyfluthrin. All CTMax percentiles for the LC10 group were higher than the corresponding values of the LC38 group (Table 1). A concentration-dependent decrease in CTMax is suggested.

Sublethal concentrations of a variety of chemicals have been shown to reduce the CTMax or upper temperature tolerance of a variety of fishes including arsenic (Paladino and Spotila 1978), cadmium (Middaugh et al. 1975; Carrier and Beitinger 1988), dieldrin (Silbergeld 1973), nickel (Becker and Wolford 1980), nitrite (Watenpaugh et al. 1985), selenium (Watenpaugh and Beitinger 1985), phenol (Chagnon and Hlohowskyj 1989), and zinc (Burton et al. 1972). In all of the above studies, chemical concentrations causing statistically significant effects were in the milligrams per litre (parts per million) range. This contrasts with cyfluthrin where CTMax reductions occurred at concentrations in the micrograms per litre (parts per billion) range.

In CTMin trials, the median temperature for fish exposed for 24 h to 96-h LC08 and LC51 concentrations increased by approximately 5.5°C, i.e., a 5.5°C loss in cold tolerance was observed. The CTMin of the cyfluthrin exposed fish was almost twice that of the control and acetone control groups. Surprisingly, there were no differences in the values of the LC08 group and those in the LC51 group. This may suggest that a threshold effect is present. That cyfluthrin effect on cold tolerance was much more dramatic than its effect on heat tolerance is not surprising. Wadleigh et al. (1991) reported that the lethality of 10 different pyrethroids (including cyfluthrin) to the german cockroach is inversely related to temperature. Kumarguru and Beamish (1981) and Coats et al. (1989) have suggested that pyrethroids in general are more toxic at lower temperatures. Our results clearly support this contention and suggest, if all else is equal, that cyfluthrin's adverse effects on nontarget species may be greater in northern climates or in cooler seasons in southern regions.

The separate effects of cyfluthrin on CTMax and CTMin are combined and illustrated in Figure 1. A zone of tolerance can be estimated as the arithmetic difference between the CTMax and CTMin. In the controls, this difference equalled 28.5°C; however, in fish exposed to the highest tested con-

centrations of cyfluthrin, this interval was only 20°C, a reduction of 30% in the temperature tolerance zone of fathead minnows.

To the best of our knowledge, this is the first reported use of the CTMin as a tool to assess the effects of a chemical in a fish. Few data are available on CTMin of fishes in general, and Beitinger and McCauley's (1990) review cited no studies where CTMin had been utilized as a bioassay for a chemical stressor. These investigators mentioned the difficulties in meeting CTMin criteria, particularly in establishing an unambiguous endpoint as test temperatures begin to approach 0°C.

In our studies, it was relatively easy to observe final loss of equilibrium at high temperatures, even with the small size of the test fish (5 – 6 mm). Loss of equilibrium was difficult to observe while fish were experiencing cooling. As the water became cooler, fish began to lie on the bottom of the beaker. Therefore, lack of response when prodded with a glass rod seemed a more appropriate endpoint for estimation of CTMin in fathead minnows. This endpoint was repeatable and unambiguous. Our endpoint may have been more difficult to determine if fish were acclimated to lower pretest temperatures which might have caused CTMin to approach 0°C. Both endpoints have been used in previous studies (Beitinger and McCauley 1990). In addition, low mortality of test fish following return to pretest conditions (5 out of 240 fish or 3%) indicates that both of our endpoints meet the criterion of critical thermal methodology. We believe that most of these deaths occurred because fish were not removed from the test tank immediately upon reaching the endpoint and hence, were exposed to more extreme temperature.

In summary, we found a 96-h LC50 of 1.08 μg cyfluthrin/L in 48-h-old fathead minnows. Exposure of larval fathead minnows for 24 h to similar sublethal cyfluthrin concentrations produced an approximate 3°C loss in heat tolerance and a 5.5°C loss in cold tolerance. The latter finding supports previous research demonstrating that the toxic effects of pyrethroids are inversely related to temperature. We speculate that sublethal effects of cyfluthrin exposure may appear at lower concentrations in northern climates.

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Computer Simulations of the Influence of Ocean Currents on Fraser River Sockeye Salmon (*Oncorhynchus nerka*) Return Times

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We hypothesized that the interannual variability of the northeast Pacific Ocean circulation affects the return times of Fraser River sockeye salmon (*Oncorhynchus nerka*). Homeward migrations were simulated for 1982 (with a relatively weak Alaska Gyre circulation) and 1983 (with a relatively strong circulation) in the context of three sequential return migration phases: a nondirected oceanic phase, a directed oceanic phase, and a directed coastal phase. Passive drifters were simulated to examine the influence of ocean currents during the nondirected oceanic phase: model fish south of 48°N were advected closer to Vancouver Island in 1983 compared with 1982; those north of 48°N were advected closer to Vancouver Island in 1982 than in 1983. Fish were simulated during the directed oceanic phase using a variety of behaviour scenarios: model fish starting south of 50°N had earlier return times in 1983 than in 1982; those starting north of 50°N had return times in 1983 that were generally the same as or later than in 1982. We inferred that ocean currents would modulate the environmental influences on return times during the directed coastal migration phase, by deflecting sockeye salmon into different oceanographic domains along the British Columbia coast.

Nous avons posé que la variabilité interannuelle de la circulation dans le nord-est de l'océan Pacifique a un effet sur le moment du retour du saumon rouge (*Oncorhynchus nerka*) du Fraser. Nous avons simulé les migrations de retour pour 1982 (circulation relativement faible du courant giratoire de l'Alaska) et pour 1983 (circulation relativement forte) dans le contexte de trois phases séquentielles : une phase océanique non dirigée, une phase océanique dirigée et une phase côtière dirigée. Nous avons employé des dériveurs passifs pour examiner par simulation l'influence des courants pendant la phase océanique non dirigée : les poissons du modèle, au sud de 48°N, étaient amenés par advection plus près de l'île de Vancouver en 1983 qu'en 1982; au nord de 48°N, ils étaient amenés par advection plus près de l'île de Vancouver en 1982 qu'en 1983. Nous avons simulé le déplacement des poissons pendant la phase océanique dirigée en suivant divers scénarios de comportement : les poissons partant du sud de 50°N revenaient plus tôt en 1983 qu'en 1982; ceux qui partaient du nord de 50°N revenaient en général en 1983 au même moment ou plus tard qu'en 1982. Nous pensons que les courants océaniques pourraient moduler les influences environnementales sur la période de retour pendant la phase de migration côtière dirigée en orientant les saumons rouges vers différents domaines océanographiques le long de la côte de Colombie-Britannique.

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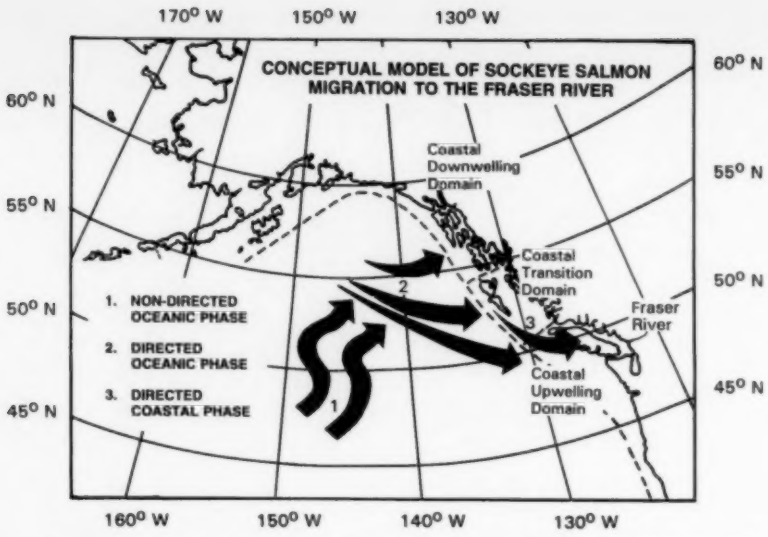

FIG. 1. Conceptual model of sockeye salmon time-sequenced return migration phases in the marine environment. Sockeye salmon swim speed, guidance mechanism, and response to environmental variables are thought to depend on the phase of return migration.

The Fraser River sockeye salmon (*Oncorhynchus nerka*) is one of British Columbia's most valuable fisheries resources, with an average annual catch (1983–86) of $60 million to Canadian fishermen and $17 million to U.S. fishermen. Reliable forecasts of the region of landfall, coastal migration route, and return times are very important to sockeye salmon fisheries management on the British Columbia coast (Healey 1993).

Sockeye salmon return migrations can be conceptualized as three time-sequenced phases: (1) a nondirected oceanic phase, (2) a directed oceanic phase, and (3) a directed coastal phase (Fig. 1). Few details are known about the ocean migration except that the directed phase is rapid, well directed, and well timed (Royce et al. 1968; French et al. 1976; Groot and Quinn 1987; Burgner 1991). Sockeye salmon coastal return migration route and return times seem to be related to atmospheric and oceanographic conditions in the winter, spring, and summer of the return year (Wickett 1977; Blackbourn 1987; Xie and Hsieh 1989; Hsieh et al. 1991).

Blackbourn (1987) proposed a temperature-displacement model to explain the interannual variation in stock-specific sockeye salmon return times. According to his model, anomalous warm winter and spring sea surface temperatures would displace sockeye salmon farther to the north than usual, requiring longer migrations to the Fraser River. Blackbourn (1987) found significant positive correlations between sea surface temperatures in the central Gulf of Alaska and return times of seven Fraser River sockeye salmon stocks.

Mysak (1986) suggested that interannual variations in the surface circulation pattern of the northeast Pacific Ocean (Fig. 2) could affect the timing and location of return migrations. Hamilton and Mysak (1986) also speculated that in years when the Sitka eddy (57°N, 135°W) is present, pink (*Oncorhynchus gorbuscha*) and sockeye salmon returning to the Nass and Skeena rivers are deflected southward by the intense anticyclonic (i.e., clockwise) vortex. Hsieh et al. (1991) found Chilko Lake sockeye salmon (a Fraser River stock) return times to be correlated with wind stress in the first marine year and sea surface temperature in the return year. However, no empirical relationship has been found between ocean surface currents in the return year and salmon run timing.

We used daily surface currents from an empirical model of the North Pacific Ocean to examine the potential influence of ocean currents. The return migrations of compass-orientated sockeye salmon were simulated for two years: 1982, with a relatively weak Alaska Gyre circulation, and 1983, with a relatively strong circulation. The influence of northeast Pacific Ocean currents on the latitude of landfall of salmon was reported in Thomson et al. (1992): the stronger circulation in 1983 deflected model fish as much as 550 km farther north than in 1982. In this paper, we examine the hypothesis that northeast Pacific Ocean surface currents affect Fraser River sockeye salmon return times.

Sockeye Salmon Migration Simulations

We investigated the potential effects of northeast Pacific Ocean surface currents on sockeye salmon, during the nondirected and directed oceanic migration phases, by seeding the Ocean Surface CURrent Simulations model (OSCURS) with arrays of passive drifters (i.e., randomly moving fish) and active drifters (i.e., compass-orientated fish).

The applicability of OSCURS to the study of sockeye salmon migration is discussed in Thomson et al. (1992); we reiterate only those aspects of the model essential to understanding our discussion here. OSCURS is an empirical model developed to examine the variability of Lagrangian drift in the North Pacific Ocean and Bering Sea (Ebbesmeyer and Ingraham 1992). It computes surface currents as the vector sum of long-term mean geostrophic currents (Fig. 2) and daily surface wind drift (Ingraham and Miyahara 1988, 1989). Most sockeye salmon in the northeast Pacific Ocean

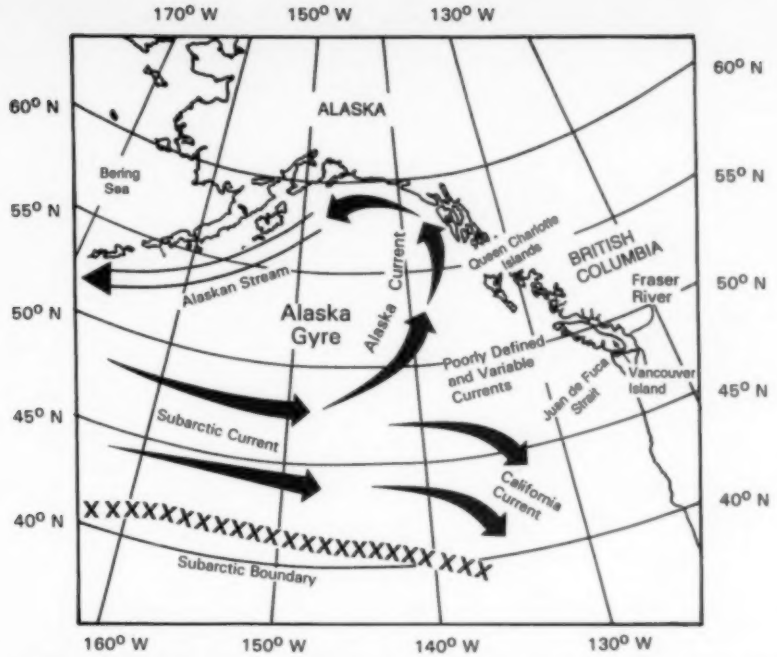

FIG. 2. Schematic of the long-term mean surface geostrophic circulation in the Gulf of Alaska (i.e., the prevailing surface currents), adapted from Dodimead et al. (1963) and Thomson (1981). Geostrophic currents are due to the slope of the sea surface, set up by the prevailing winds. These surface currents have magnitudes of about 5–10 km·d^{-1} in the Subarctic Current, 30 km·d^{-1} in the Alaska Current, 20 km·d^{-1} in the California Current, and over 100 km·d^{-1} in the Alaskan Stream. East of the Subarctic Current bifurcation, the ocean currents are poorly defined and variable. The southern limit of the Alaska Gyre is delineated by the Subarctic Boundary, a salinity front that has been frequently referred to as the southern limit of salmon distributions in the northeast Pacific Ocean.

are found in the upper 15 m of the water column (French et al. 1976; Burgner 1991). In this surface layer, the instantaneous wind-driven currents are stronger than the mean geostrophic flow, and tracks of surface drifters (i.e., drogued at 20 m depth) tend to follow time-averaged isobars of atmospheric pressure (Emery et al. 1985).

Our simulations focused on 1982 and 1983, years with representative weak and strong Alaska Gyre circulation patterns, respectively. For each simulation, 174 drifters were used (Fig. 3). Passive drifters were simulated to examine the potential influence of currents on randomly moving fish during the nondirected oceanic migration phase. For each year, the trajectories of passive drifters were simulated with start dates of 1 May, 1 June, and 1 July. Active drifters were simulated to examine the potential effects of currents on sockeye salmon during the directed oceanic migration phase. Compass-orientated fish were simulated with 27 behaviour scenarios using combinations of swim speed (18, 30, and 48 km·d^{-1}), compass orientation (90°T (east), 112.5°T (east–southeast), and 135°T (southeast)), and migration start date (1 May, 1 June, and 1 July). The rationale for choosing these behaviour scenarios and the array of migration start locations is described in Thomson et al. (1992).

The return date of arrival off Juan de Fuca Strait (Fig. 2) was calculated for each active drifter, using the simulated date and latitude of landfall (landfall was assumed to occur when a model fish reached the continental shelf) and a constant migration rate along the coast. Sockeye salmon, during their homeward migration, take about 2 wk to travel along the coast from the Queen Charlotte Islands to Juan de Fuca Strait and 1 wk to travel the length of Vancouver Island (D. Blackbourn and J. Woodey, personal communication). On the basis of this information, we used a coastal migration rate of 38.7 km·d^{-1}, which compares well with ultrasonic tag studies of sockeye salmon along the south coast of British Columbia (Quinn 1988).

Mean differences in return dates between 1983 and 1982 were calculated for simulated sockeye salmon originating from the six areas shown in Fig. 3. The 95% confidence limits of the population mean were used to determine whether or not the sample mean return time differed significantly between the two years.

Migration Simulation Results

The simulated passive drifters moved to the northeast around the Alaska Gyre, for the most part. Figure 4 shows the difference in passive drifter trajectories between 1982 and 1983, as well as the meandering effects of wind-driven currents. The stronger currents in 1983, compared with 1982, would advect passive drifters farther around the Alaska Gyre. Simulated drifters started south of 48°N were advected closer to Vancouver Island in the first 2 mo of ocean drift in 1983, compared with 1982. However, simulated drifters

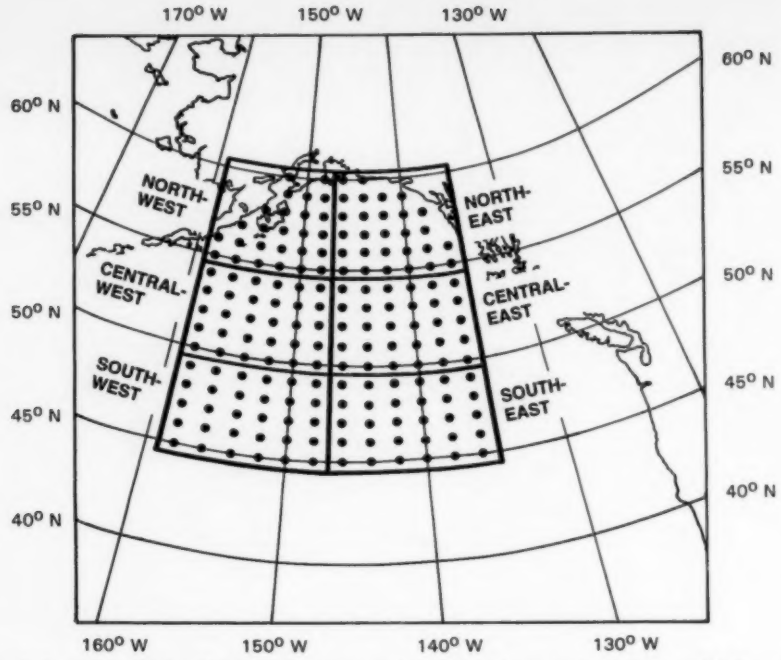

FIG. 3. Locations of the 174 migration start positions used in each simulation, and delineation of the six geographic areas referenced in Tables 1–4.

started north of 48°N were advected closer to Vancouver Island in 1982 than in 1983.

The trajectories of simulated active drifters (i.e., simulated compass-orientated sockeye salmon) made landfall earlier and farther north in 1983, compared with 1982. In Fig. 5, for example, the simulated sockeye salmon, started at 50°N, arrived on the coast about 2 wk earlier and about 400 km farther to the north in 1983 than in 1982. Although the model fish arrived at the coast earlier in 1983, a longer coastal swim to the Fraser River would be required than in 1982; a migration rate of about 35 km·d^{-1} along the coast would result in no difference in return time to Juan de Fuca Strait between the two years.

The effect of ocean currents on simulated return times, during the directed oceanic phase, was strongly dependent on the geographic location from which model fish were started (Table 1). Simulated sockeye salmon originating from the southwest and southeast areas arrived off Juan de Fuca Strait nearly 1 wk earlier in 1983 compared with 1982, model fish from the central–east area arrived about 1 d earlier in 1983, and model fish from the northwest, northeast, and central–west areas arrived at about the same time in 1983 and 1982.

The influence of currents on simulated return times was also dependent on swim speed, compass orientation, and migration start date. The differences in mean return times between the two years decreased with increased swim speed (Table 2). With an 18 km·d^{-1} swim speed, model fish from the southwest and southeast areas arrived about 1.5 wk earlier in 1983 compared with 1982; with a 48 km·d^{-1} swim speed, these simulated sockeye salmon arrived about 2 d earlier in 1983.

Compass orientation affected the mean return time differences between 1983 and 1982 (Table 3); however, there was no discernable relationship between orientation and return time differences. Simulated sockeye salmon with an eastward orientation, starting from the southwest and southeast areas, arrived almost 1 wk earlier in 1983 (compared with 1982), and model fish from the central–west area arrived a day or so later in 1983. Simulated sockeye salmon with an east–southeastward orientation, starting from the central–west and central–east areas, arrived a few days earlier in 1983 than in 1982, and model fish from the southeast area arrived almost 1 wk earlier in 1983. Model fish with a southeastward orientation did not differ in return time.

Migration start date affected return time differences between the two years (Table 4); however, there was no discernable relationship between migration start date and return time differences. For a migration start date of 1 May, simulated sockeye salmon arrived at Juan de Fuca Strait about 1.5 wk earlier in 1983 when starting from the southwest area, a day or so earlier when starting from the northeast area, and several days later when starting from the northwest area. For a 1 June migration start, model fish arrived about 1.5 wk earlier in 1983 when starting from the southeast area, about 0.5 wk earlier when starting from the southwest area, and about 2 d earlier when starting from the central–east area. From the areas to the north and west, the model fish starting 1 June arrived several days later in 1983 than in 1982. Simulated sockeye salmon from all areas with the exception of the central–east, starting their migration on 1 July, arrived up to several days earlier in 1983.

Discussion

The simulation results illustrate that the interannual variability of northeast Pacific Ocean surface currents would

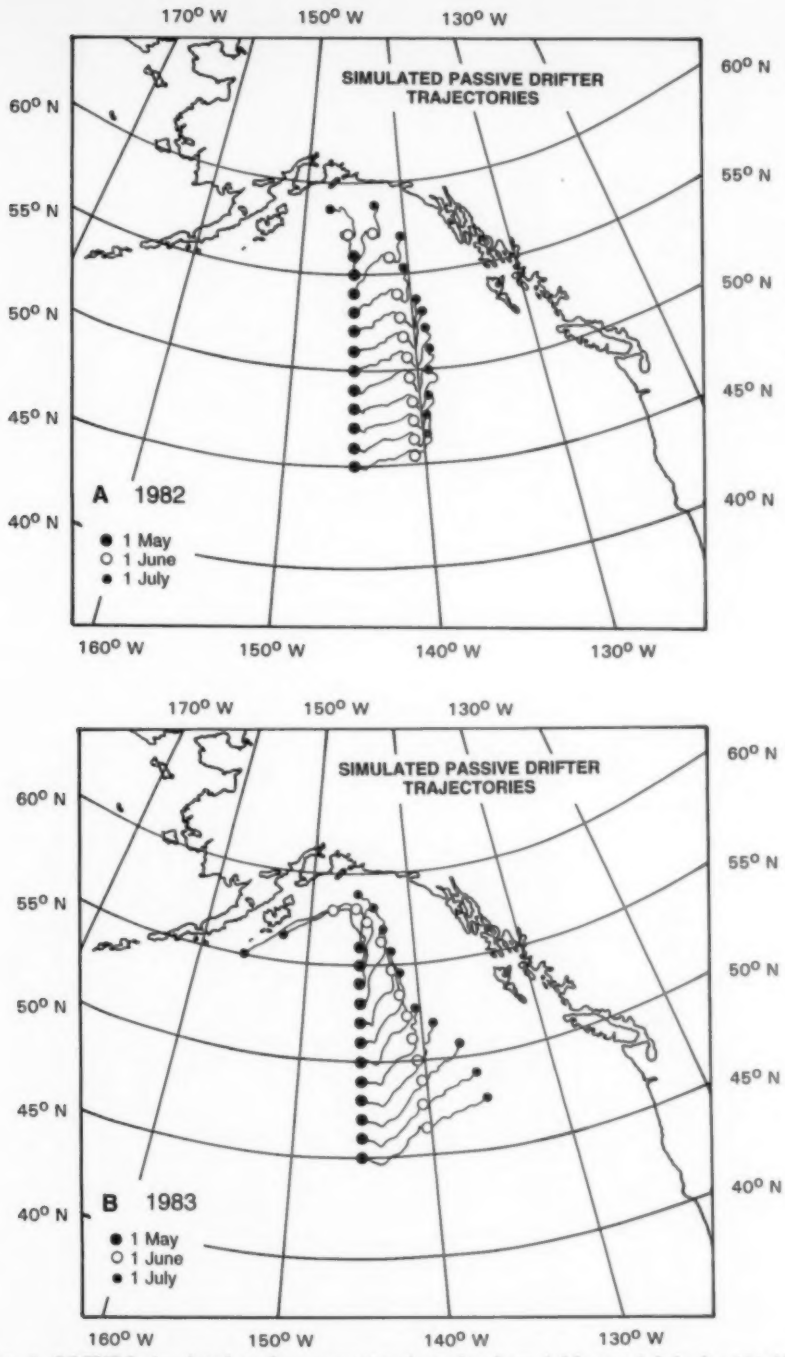

FIG. 4. OSCURS-simulated surface current trajectories from 1 May to 1 July for (A) 1982 and (B) 1983.

affect the return times of Fraser River sockeye salmon by up to 1.5 wk (i.e., three times the standard deviations of estimated annual peak return dates of seven Fraser River stocks (Blackbourn 1987)). To provide a perspective on the importance of these differences, we note that a few days' difference in the return time of any Fraser River sockeye salmon stock is sufficient to impact the fisheries management plan and the commercial sockeye salmon fisheries along the British Columbia coast (D. Blackbourn, personal communication).

Unfortunately, we cannot compare run timing differences between our 1982 and 1983 simulations with differences between actual 1982 and 1983 timing data because there

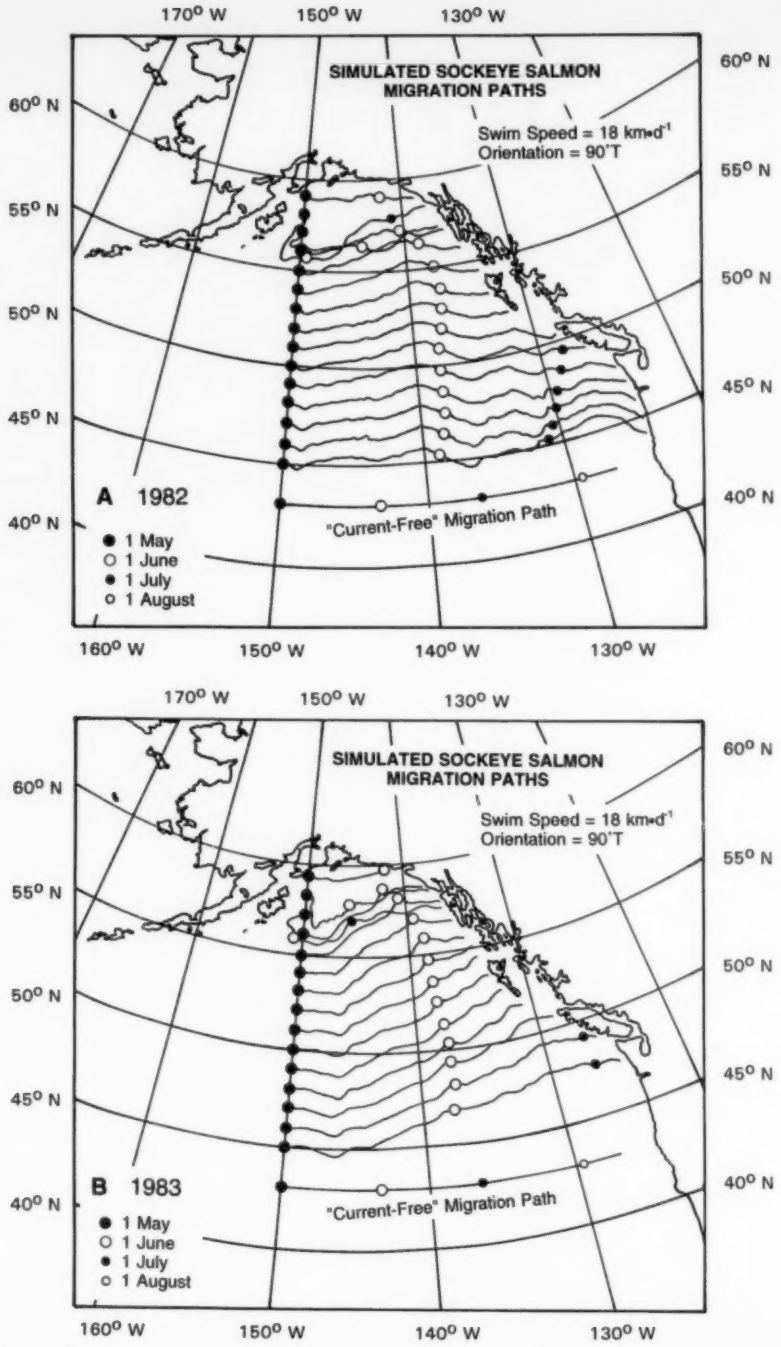

FIG. 5. Simulated sockeye salmon migration paths for (A) 1982 and (B) 1983, with a swim speed of 18 km·d^{-1}, a compass orientation of 90°T, and a migration start date of 1 May. Departures from "current-free" migration paths (a sample is shown) and meanders are due solely to the time- and space-varying surface currents.

are no run timing data available for 1983 due to the high Northern Diversion Rate (defined as the percentage of sockeye salmon returning to the Fraser River around the north end of Vancouver Island rather than around the south end of the island through Juan de Fuca Strait). Regardless, the simulation results permit an examination of the kinematics of ocean circulation effects on sockeye salmon return times in the context of the three return migration phases (Fig. 1). During

TABLE 1. Mean difference in return date between 1983 and 1982 (in days), using all 27 behaviour scenarios. Statistics are provided for each geographic area shown in Fig. 3. Mean differences in bold type are significantly different from zero. The bracketed values are the 95% confidence interval of the mean; n is sample size. Simulated sockeye salmon that made landfall south of 45°N are not included. A negative (positive) mean indicates that the return dates in 1983 were earlier (later) than in 1982.

Northwest:	0.4 (−0.4, 1.2) n = 471	Northeast:	−0.2 (−0.6, 0.2) n = 711
Central–west:	0.1 (−0.6, 0.8) n = 423	Central–east:	**−0.6** (−1.1, 0.1) n = 541
Southwest:	**−6.1** (−7.1, −5.1) n = 196	Southeast:	**−5.3** (−6.2, −4.4) n = 199
Total area:	**−1.0** (−1.3, −0.7), n = 2541		

TABLE 2. Mean difference in return date between 1983 and 1982 (in days) for three simulated swim speeds, using all orientations and migration start dates. Statistics are provided for each geographic area shown in Fig. 3. Mean differences in bold type are significantly different from zero. The bracketed values are the 95% confidence interval of the mean; n is sample size. Simulated sockeye salmon that made landfall south of 45°N are not included. A negative (positive) mean indicates that the return dates in 1983 were earlier (later) than in 1982.

	Swim speed = 18 km·d^{-1}		
Northwest:	1.2 (−1.0, 3.3) n = 150	Northeast:	−0.5 (−1.6, 0.5) n = 242
Central–west:	−0.5 (−2.2, 1.2) n = 150	Central–east:	**−1.6** (−3.0, −0.2) n = 177
Southwest:	**−10.6** (−12.8, −8.4) n = 61	Southeast:	**−10.1** (−12.1, −8.1) n = 57
Total area:	**−1.8** (−2.5, −1.1), n = 837		
	Swim speed = 30 km·d^{-1}		
Northwest:	0.5 (−0.6, 1.5) n = 161	Northeast:	0.0 (−0.6, 0.6) n = 234
Central–west:	0.3 (−0.8, 1.3) n = 135	Central–east:	−0.3 (−1.0, 0.4) n = 186
Southwest:	**−6.4** (−7.8, −5.1) n = 63	Southeast:	**−4.6** (−5.9, −3.3) n = 68
Total area:	**−0.8** (−1.2, −0.4), n = 847		
	Swim speed = 48 km·d^{-1}		
Northwest:	−0.4 (−0.9, 0.1) n = 160	Northeast:	−0.1 (−0.4, 0.2) n = 235
Central–west:	**0.5** (0.1, 1.0) n = 138	Central–east:	0.1 (−0.4, 0.6) n = 178
Southwest:	**−1.9** (−2.6, −1.4) n = 72	Southeast:	**−2.2** (−3.2, −1.4) n = 74
Total area:	**−0.4** (−0.6, −0.2), n = 857		

the nondirected oceanic phase, randomly moving fish would be advected by surface currents within the Alaska Gyre (Fig. 2). The Subarctic Current would advect sockeye salmon to the east, the Alaska Current would advect sockeye salmon to the northeast and around the top of the Alaska Gyre, and the Alaskan Stream would advect sockeye salmon towards the southwest. Our simulations show that randomly moving fish would be advected closer to (or farther away from) the Fraser River, depending on their location within the gyre and the interannual variability of the currents; this would affect sockeye salmon return times by requiring shorter (or longer) travel distances in the directed oceanic and coastal migration phases.

The effects of ocean currents on simulated compass-orientated sockeye salmon, during the directed oceanic phase, were dependent on swim speed, compass orientation, migration start date, and, most importantly, premigration position. The Subarctic Current would assist sockeye salmon in their homeward migration dependent on the magnitude of the current in the surface layer: the stronger Alaska Gyre circulation in 1983, compared with 1982, resulted in earlier return times for model fish starting the directed oceanic migration phase within the Subarctic Current. Simulated sockeye salmon starting the directed return migrations in the Alaska Current or Alaskan Stream had return times in 1983 that were the same as or later than return times in 1982 due to (1) large northward deflections in the Alaska Current requiring longer migrations to the Fraser River within the coastal environment (Thomson et al. 1992) or (2) increased westward transport in the Alaskan Stream which impeded the eastward migration.

Our simulations imply that ocean currents would modulate the influence of the coastal environment on sockeye salmon run timing by deflecting fish into different marine environments along the British Columbia coast (e.g., see Fig. 1: Coastal Downwelling Domain, Coastal Transition Domain, and Coastal Upwelling Domain (Ware and MacFarlane 1989)). Sockeye salmon migrating through the Alaska Current would be deflected to the northeast with magnitudes depending on the year-to-year strength of the surface currents: the stronger Alaska Gyre circulation of 1983, compared with 1982, placed our model fish on the coast farther to the north (Thomson et al. 1992), thereby requiring longer migrations to the Fraser River within the complex coastal environment (Thomson et al. 1989; Crawford and Thomson 1991; Jardine et al. 1993).

Our examination of the effects of ocean currents assumed that sockeye salmon are found in the same locations, each year, at the beginning of the directed and nondirected oceanic migration phases. This is probably not the case: sockeye salmon may have stock-specific distributions in the northeast Pacific Ocean, and sockeye salmon distributions may be displaced to the north (south) by anomalous warm (cold) sea surface temperatures (Blackbourn 1987). However, the run timing effects of spring/summer ocean currents on stock-specific sockeye salmon distributions, affected by winter/spring sea surface temperatures, can be inferred. The influ-

TABLE 3. Mean difference in return date between 1983 and 1982 (in days) for three simulated orientations, using all swim speeds and migration start dates. Statistics are provided for each geographic area shown in Fig. 3. Mean differences in bold type are significantly different from zero. The bracketed values are the 95% confidence interval of the mean; *n* is sample size. Simulated sockeye salmon that made landfall south of 45°N are not included. A negative (positive) mean indicates that the return dates in 1983 were earlier (later) than in 1982.

Compass orientation = 90°T (eastward)			
Northwest:	0.4 (−0.8, 1.6) *n* = 232	Northeast:	0.0 (−0.3, 0.3) *n* = 252
Central–west:	**1.2** (0.6, 1.9) *n* = 270	Central–east:	0.5 (−0.1, 1.0) *n* = 270
Southwest:	**−6.1** (−7.1, −5.1) *n* = 195	Southeast:	**−5.3** (−6.3, −4.4) *n* = 182
Total area:	**−1.2** (−1.5, −0.8), *n* = 1401		
Compass orientation = 112.5°T (east–southeastward)			
Northwest:	0.5 (−0.5, 1.5) *n* = 223	Northeast:	0.0 (−0.4, 0.4) *n* = 252
Central–west:	**−1.9** (−3.5, −0.4) *n* = 153	Central–east:	**−2.1** (−3.0, −1.1) *n* = 242
Southwest:	*n* = 0	Southeast:	**−5.3** (−8.3, −2.3) *n* = 17
Total area:	**−0.9** (−1.3, −0.4), *n* = 887		
Compass orientation = 135°T (southeastward)			
Northwest:	−0.8 (−9.7, 8.1) *n* = 16	Northeast:	−0.7 (−2.0, 0.5) *n* = 207
Central–west:	*n* = 0	Central–east:	1.7 (−2.7, 6.2) *n* = 29
Southwest:	*n* = 0	Southeast:	*n* = 0
Total area:	−0.5 (−1.7, 0.8), *n* = 252		

TABLE 4. Mean difference in return date between 1983 and 1982 (in days) for three simulated migration start dates, using all swim speeds and orientations. Statistics are provided for each geographic area shown in Fig. 3. Mean differences in bold type are significantly different from zero. The bracketed values are the 95% confidence interval of the mean; *n* is sample size. Simulated sockeye salmon that made landfall south of 45°N are not included. A negative (positive) mean indicates that the return dates in 1983 were earlier (later) than in 1982.

Migration start date: 1 May			
Northwest:	**3.2** (1.6, 4.9) *n* = 153	Northeast:	**−1.3** (−1.9, −0.8) *n* = 239
Central–west:	0.0 (−1.5, 1.5) *n* = 142	Central–east:	−0.3 (−1.3, 0.6) *n* = 178
Southwest:	**−10.2** (−12.2, −8.1) *n* = 68	Southeast:	−1.3 (−3.0, 0.4) *n* = 60
Total area:	**−0.8** (−1.3, −0.2), *n* = 840		
Migration start date: 1 June			
Northwest:	**1.6** (0.3, 2.8) *n* = 160	Northeast:	**1.7** (1.1, 2.3) *n* = 240
Central–west:	**3.0** (2.3, 3.7) *n* = 161	Central–east:	**−1.9** (−3.1, −0.8) *n* = 182
Southwest:	**−4.2** (−5.3, −3.0) *n* = 82	Southeast:	**−10.8** (−11.9, −9.6) *n* = 73
Total area:	−0.4 (−0.9, 0.1), *n* = 898		
Migration start date: 1 July			
Northwest:	**−3.5** (−4.5, −2.5) *n* = 158	Northeast:	**−1.0** (−1.9, −0.1) *n* = 232
Central–west:	**−3.8** (−5.0, −2.6) *n* = 120	Central–east:	0.5 (−0.1, 1.1) *n* = 181
Southwest:	**−3.5** (−4.6, −2.5) *n* = 46	Southeast:	**−2.9** (−3.6, −2.4) *n* = 66
Total area:	**−1.9** (−2.3, −1.5), *n* = 803		

ence of ocean temperatures is consistent over the northeast Pacific Ocean (relatively warm temperatures would force relatively late return times) whereas the influence of ocean currents is due primarily to the Subarctic Current, and a relatively strong Alaska Gyre circulation would force relatively early return times for sockeye salmon stocks in the vicinity of the Subarctic Current (Fig. 2). For stocks in the northern portion of the Alaska Gyre (e.g., Adams River stocks; Blackbourn 1987), the effects of currents on run timing would be minimal. For stocks in the vicinity of the northern boundary of the Subarctic Current (e.g., Horsefly River and Chilko River stocks; Blackbourn 1987), the effects of ocean currents on run timing would augment the effects of ocean temperatures: warm temperatures would displace sockeye salmon northward away from the eastward drift of the Subarctic Current and cold temperatures would displace fish southward into the eastward flow.

The effects of ocean currents on stocks within the Subarctic Current (e.g., Early Stuart Lake stocks; Blackbourn 1987) would depend on whether cold (warm) winter/spring temperatures lead strong (weak) spring/summer currents. If a strong (weak) Alaska Gyre circulation follows warm (cold) ocean temperatures, then the effects of currents on return times would tend to offset the effects of surface temperatures; however, if a weak (strong) Alaska Gyre circulation follows warm (cold) temperatures, the eastward drift of the Subarctic Current would reinforce the effects of ocean temperatures on run timing.

Would sockeye salmon using bicoordinate navigation, rather than compass orientation as we have modelled, be affected by ocean currents? Consider an aircraft attempting to navigate to an airport without a forecast of winds perpendicular to the intended route: the aircraft would reach its goal, but it would be deflected from the intended flight path regardless of navigational precision, requiring a longer period of time to reach its goal or more fuel to travel faster. Navigating sockeye salmon would be similarly affected by ocean currents; they would be deflected from their intended migration route and arrive later or require more energy to reach their goal at the intended time. To migrate along a "straight" line in the presence of ocean currents, sockeye salmon would require a positive rheotactic response (i.e., orientation into oncoming currents) in addition to compass orientation or bicoordinate navigation; we expect that ocean currents would still affect salmon return times or bioenergetics. The direction-finding mechanism used by sockeye salmon during the return migrations is unknown (Healey and Groot 1987; Quinn 1990). We suggest that interannual variations in northeast Pacific Ocean currents would affect sockeye salmon return times, regardless of the direction-finding mechanism used.

The results from our simulations are consistent with the hypothesis that ocean currents affect the return times of homeward migrating sockeye salmon. Since we examined only two years with insufficient run timing data available for verification, this must be considered a conceptual model. Incorporation of stock-specific effects of ocean currents on return times into fisheries forecasts may provide more reliable empirical relations for the management of Fraser River sockeye salmon stocks. An examination of ocean current and run timing data over many more years (i.e., 1950 to present) is required.

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Diel Feeding and Positioning Periodicity of a Grazing Mayfly in a Trout Stream and a Fishless Stream

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We studied the feeding and positioning periodicity on natural substrates of the overwintering and fast-growing summer generations of a grazing mayfly, *Baetis bicaudatus*, in a third-order trout stream and a fishless, first-order tributary in western Colorado. At 4-h intervals over 24-h, we recorded the number of *Baetis* on stone tops in flow-through enclosures in situ and in streamside circular flow-through chambers. We determined the feeding periodicity of *Baetis* using abundance of plant pigments as an index of gut fullness. *Baetis* were nocturnal in the trout stream; more animals were found on stone tops and guts were fuller at night, suggesting that individuals came to stone tops to feed during darkness. However, *Baetis* from the fishless stream were either aperiodic or weakly nocturnal. We tested the hypothesis that nocturnal feeding by *Baetis* is a response to the presence of visually feeding trout by conducting transplant experiments in the circular streams. After 24 h, *Baetis* transferred from the trout stream to fishless water remained strongly nocturnal, while *Baetis* transferred from the fishless stream to trout water became significantly more nocturnal, suggesting that the risk of fish predation outweighs the benefits of relaxing nocturnal periodicity to feed continuously.

Nous avons étudié la périodicité de l'alimentation et de la position, sur les substrats naturels de générations de larves brouteuses d'éphémères, *Baetis bicaudatus*, qui hivernent et à croissance rapide en été, dans un cours d'eau salmonicole de troisième ordre et un affluent de premier ordre dans poisson, dans l'ouest du Colorado. À intervalle de 4 h pendant une période de 24 h, nous avons enregistré le nombre de *Baetis* sur la face exposée des pierres dans des enceintes in situ à écoulement continu, et dans des enceintes circulaires à écoulement continu en bordure de cours d'eau. Nous avons déterminé la périodicité de l'alimentation des *Baetis* par l'abondance de pigments végétaux comme indice de réplétion du tube digestif. Ces organismes étaient nocturnes dans le ruisseau salmonicole; on a trouvé plus d'animaux sur la face exposée des pierres et le tube digestif était plus rempli la nuit, ce qui semble indiquer que les individus viennent sur la face exposée des pierres pour se nourrir pendant la période d'obscurité. Toutefois, les *Baetis* des cours d'eau sans poisson étaient apériodiques ou faiblement actifs la nuit. Nous avons vérifié l'hypothèse selon laquelle l'alimentation nocturne de ces organismes est une réponse à la vue de truites qui se nourrissent en effectuant des expériences de transplantation dans des cours d'eau circulaires. Après 24 h, des *Baetis* transférés du cours d'eau salmonicole dans un cours d'eau sans poisson conservaient leurs habitudes d'alimentation nocturne, tandis que les organismes transférés du cours d'eau sans poisson dans un cours d'eau salmonicole étaient nettement plus actifs la nuit, ce qui semble indiquer que le risque de prédation par les poissons l'emporte sur les avantages que présente une alimentation en continu sans périodicité nocturne.

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Mayfly periodicity has been widely studied, initially in the context of diel drift periodicity (Waters 1972). Interest has recently shifted from the study of drift patterns to processes or mechanisms, with an emphasis on resolving the question of whether drift is an active behavioral phenomenon or a passive dislodgment into the water column. To answer this question, most investigators have attempted to demonstrate concordance (implicating passive drift) or nonconcordance (suggesting an active mechanism) of mayfly feeding or activity periodicity with diel drift patterns (Bailey 1981; Kohler 1983; Allan et al. 1986, 1991; Casey 1987; Rader and Ward 1990; Wilzbach 1990). Reviews of the feeding and activity periodicity of the grazing mayfly nymph, *Baetis* spp. (Ephemeroptera: Baetidae), indicate that data are often contradictory (Rader and Ward 1990; Wilzbach 1990; Allan et al. 1991), due in part to the great diversity in methodologies, species, and stream conditions used. *Baetis* has been considered nocturnal (Elliott 1968; Kovalak 1978; Ploskey and Brown 1980; Casey 1987; Glozier and Culp 1989), diurnal (Graesser and Lake 1984; Statzner and Mogel 1985; Allan et al. 1986; Wilzbach 1990), and aperiodic (Kovalak 1979; Kohler 1983, 1985 (in part)). Occasionally,

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TABLE 1. Summary of *Baetis* periodicity studies conducted in the East River (fish) and Benthette Brook (no fish).

Study, site, and date	Replication (*n*)	Sampling schedule	*Baetis* cohort
1. Substrate use			
No fish, July 17–18, 1985	6	Every 4 h beginning at 13:00	Winter
No fish, Aug. 14–15, 1985	6	Every 4 h beginning at 13:00	Summer
Fish, Sept. 1–2, 1987	6	Every 4 h beginning at 13:00	Summer
2. Microhabitat use (circular streams)			
No fish, June 17–18, 1991	7	Every 4 h beginning at 13:00	Winter
Fish, June 22–23, 1991	7	Every 2 h (day) or 4 h (night)	Winter
3. Feeding periodicity			
No fish, June 12–13, 1990	12	Every 3 h beginning at 9:00	Winter
No fish, July 29–30, 1990	10	Every 3 h beginning at 15:00	Summer
No fish, Aug. 6–7, 1989	10	6:00, 8:00, 10:00, 14:00, 18:00, 20:00, 22:00, 2:00	Summer
Fish, June 18–19, 1990	12	Every 3 h beginning at 9:00	Winter
Fish, July 14–17, 1989	15	Every 2 h beginning at 8:00	Winter
Fish, Aug. 13–14, 1991	10	Every 3 h beginning at 9:00	Summer

results within studies have been contradictory as well (Allan et al. 1991).

In our research with *Baetis bicaudatus* Dodds and its stonefly predators (e.g., Peckarsky and Penton 1989; Peckarsky 1991), we needed to know the times of day both predator and prey were most active, in order to schedule and interpret behavioral experiments in our two study streams. Because generalization from the literature was difficult, we examined the periodicity of *Baetis bicaudatus* feeding and substrate use in three types of experiments between July 1985 and August 1991. Preliminary results from these studies indicated that *Baetis* periodicity differed in the two streams, one of which is a third-order trout stream and the other its fishless tributary. To further investigate differences in periodicity that may be related to the presence or absence of fish, we began in summer 1989 to incorporate a comparison of *Baetis* periodicity in the two streams into the experimental design. In addition, transplant studies were conducted to test whether the periodicity of *Baetis* from each stream was behaviorally fixed or flexible. Experiments were conducted several times throughout the summer to compare the periodicity of the summer and winter cohorts of this bivoltine species, differences between which were also suggested in early results.

The objectives of this study were to (1) determine the periodicity of *Baetis bicaudatus* feeding and substrate use in two Rocky Mountain streams, (2) compare *Baetis* periodicity in a trout stream and a fishless stream and at different times throughout the summer, and (3) determine whether periodicity can be modified over the short term by transferring individuals from one stream into water from the other.

Methods

Study Site

Experiments were carried out on *Baetis bicaudatus* from the East River, a third-order, high-altitude (2850 m) trout stream in the Colorado Rocky Mountains (Gunnison Co.) (Peckarsky 1979), and Benthette Brook, a fishless, first-order tributary of the East River (Peckarsky and Penton 1989). At this elevation on the East River, native brook (*Salvelinus fontinalis*) and stocked rainbow trout (*Oncorhynchus mykiss*) are abundant, with smaller numbers of brown (*Salmo trutta*) and cutthroat trout (*Oncorhynchus clarki*, previously *Salmo clarki*) (Allan 1981). A 50-m waterfall at the mouth of Benthette Brook provides a barrier to colonization by fish, rendering this small tributary naturally fishless.

Baetis Life History

Baetis bicaudatus is abundant in the East River and Benthette Brook (Peckarsky 1991) and is bivoltine in both streams (B.L. Peckarsky, unpublished data). The first (winter) cohort overwinters as larvae and then emerges, mates, and oviposits from late June through July. The second (summer) cohort hatches from those eggs and grows rapidly, emerging and reproducing throughout August and September. The two cohorts overlap in July, but summer cohort larvae can be distinguished by their small size relative to wing pad development, by the relatively smaller final size of the last instar nymphs, and by the presence of a third, often minute, caudal filament (B.L. Peckarsky, unpublished data). By early August, most baetids in either stream are of the summer cohort or are the minute, early instars of the next overwintering generation.

Periodicity of Substrate Use

Visual observations of *Baetis* substrate use were made in Benthette Brook in July and August 1985 and in the East River in September 1987 (Table 1) to determine whether relative abundance of larvae on stone tops varied over a 24-h period. Fifteen *Baetis* were placed in 10 × 10 × 25 cm flow-through (800-μm-mesh ends) Plexiglas enclosures on the stream bed containing 10 stones each: three of 8-cm, three of 6-cm, and four of 4-cm diameter (Peckarsky and Penton 1989). At 4-h intervals, for 24-h beginning at 13:00 (MDT), the number of *Baetis* visible on stones from above each enclosure was recorded. Red light was used to locate individuals at night, which did not appear to influence behavior or cause dispersal (Allan et al. 1986, but see Heise 1992). To determine whether substrate use differed between daylight and darkness, paired *t*-tests were performed for each date on the mean percentage of animals visible on

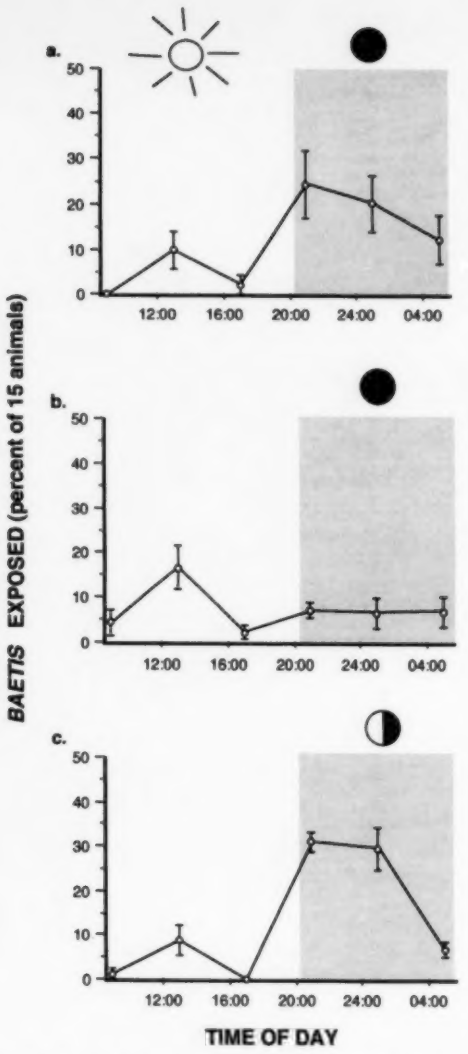

FIG. 1. Percentage of 15 *Baetis* on stone tops in flow-through enclosures in the substrate use experiments. (a) Fishless stream, July 1985; (b) fishless stream, August 1985; (c) trout stream, September 1987. Values are means ± SE. Shaded area indicates hours of darkness; moon phase is indicated above the shading.

stones in each box during daylight (9:00, 13:00, 17:00) and nighttime (21:00, 1:00, 5:00). An arc sine square-root transformation was applied to normalize percentage data and stabilize variances. Decision rules for defining periodicity in these and all other experiments were as follows: nocturnal: response variable greater at night than during the day; diurnal: night < day; and aperiodic: night not different from day. Where the relative strength of periodicity is discussed (i.e., data described as "more weakly" or "more strongly" nocturnal than another set), these comparisons are based on the magnitude of the difference between nighttime and daytime values, the value of t, and the probability associated with t. Because water temperature, as well as light, may potentially play a role in periodicity, the strength of correlation between temperature and periodicity of substrate use was measured using Spearman rank correlation analysis.

Microhabitat Use

A more precise version of the substrate use experiments was conducted in June 1991 (Table 1) to gain more detailed information about *Baetis* microhabitat use and periodicity and to compare periodicity of *Baetis* from the East River with those from Benthette Brook. These studies were conducted in a streamside system of 15-cm-diameter circular Plexiglas chambers (Peckarsky and Cowan 1991) supplied by a continuous supply of fresh stream water from either the East River or the fishless tributary, Benthette Brook. Water did not recirculate, so that animals in the chambers experienced natural thermal and chemical regimes. The stream units were housed in a translucent vinyl weatherport and were thereby exposed to natural fluctuations in light intensity. Each stream chamber contained four 4-cm-diameter periphyton-covered stones and maintained current velocities of 15–25 cm/s.

On June 17–18, 1991, five *Baetis* from Benthette Brook were placed in each of seven replicate chambers and observed by instantaneous scan sampling every 4 h for 24 h, beginning at 13:00 (Table 1); the streams were supplied with Benthette Brook water. The design of the chambers afforded a clear view of both the tops and sides of stones and occasionally the bottoms. In addition, the circular shape of the streams enabled animals to drift and resettle more naturally without becoming entrained on a downstream screen, as in the unidirectional, flow-through enclosures. Consequently, the number of animals drifting, as well as the number on each stone surface (top, upstream side, downstream side), was recorded by eye at each observation. Red light was used to observe animals at night. The experiment was repeated on June 22–23, 1991, using *Baetis* and stream water from the East River. Animals were observed every 2 h during the day and every 4 h at night (Table 1). Data were analyzed by paired t-test and Spearman rank correlation, as described previously, using percentage on tops of stones (no. on tops/no. on stones) as the response variable. Drifting animals, and animals otherwise not on stones, were excluded from the analysis for each observation, resulting occasionally in tank totals of less than five animals, particularly at night when drift was most prevalent.

Feeding Periodicity

A third study was designed to determine *Baetis* feeding periodicity in the East River and Benthette Brook and was repeated to facilitate comparisons between streams and across seasons (winter versus summer cohort *Baetis*). Ten to 15 *Baetis* (depending on the study) were obtained from benthic samples in a single riffle at regular intervals over 24 h (see Table 1 for sampling dates, intervals, and replication). Upon sampling, animals were placed individually in amber bottles containing several millilitres of 90% ethanol, and gut fullness was determined fluorometrically (Cowan and Peckarsky 1990). Gut pigments were extracted from macerated animals and the fluorescence of the extracts read on a Turner model 112 fluorometer. Gut fullness, or the amount of algal material in the gut at the time of sampling, was expressed as nanograms of total pigment (chlorophyll *a* + phaeopigment *a*) per *Baetis*. Data were analyzed by two-sample t-test, comparing log-transformed night versus day gut

TABLE 2. Results of statistical analyses of *Baetis* periodicity studies in Benthette Brook (no fish) and the East River (fish). Paired *t*-tests were performed on night and day differences in percentage of *Baetis* using stone tops in the substrate use and microhabitat studies. Two-sample *t*-tests compared log-transformed night and day gut fullness (ng pigment per individual) in the feeding periodicity studies.

Study, site, and date	Mean difference (% night − % day) ± 1 SE		t	$p > t$	Conclusion
1. Substrate use					
No fish, July 1985	15.0 ± 4.1		3.65	0.015	Nocturnal
No fish, Aug. 1985	−0.92 ± 0.94		−0.98	0.374	Aperiodic
Fish, Sept. 1987	19.2 ± 1.9		10.0	0.0002	Nocturnal
2. Microhabitat use					
No fish, June 1991	28.1 ± 6.3		4.13	0.006	Nocturnal
Fish, June 1991	51.9 ± 5.9		13.04	0.001	Nocturnal
Fish, Aug. 1991	31.9 ± 6.8		7.47	0.003	Nocturnal
3. Feeding periodicity	Nighttime (mean ± 1 SE)	Daytime (mean ± 1 SE)			
No fish, June 1990	148.6 ± 15.4	87.8 ± 10.7	2.70	0.008	Nocturnal
No fish, July 1990	88.7 ± 8.2	66.6 ± 8.8	2.54	0.013	Nocturnal
No fish, Aug. 1989	132.4 ± 13.8	148.4 ± 16.4	−0.13	0.898	Aperiodic
Fish, June 1990	91.1 ± 12.5	38.1 ± 4.0	4.06	0.0001	Nocturnal
Fish, July 1989	133.6 ± 11.1	48.5 ± 4.7	7.46	0.0001	Nocturnal
Fish, Aug. 1991	89.7 ± 8.4	68.6 ± 9.3	2.18	0.032	Nocturnal

fullness on each sampling date. Spearman rank correlations were performed on temperature and gut fullness over 24 h.

Gut Clearance Time

Inferences about feeding rates can be made from gut fullness data only if the gut clearance time is known because ingestion rate is the product of instantaneous gut fullness and the gut clearance rate constant (Boyd et al. 1980; Dagg and Wyman 1983). The sampling interval in diel feeding periodicity studies should be no less than the gut clearance time of the study animal (Mackas and Bohrer 1976). Otherwise, full guts may simply be an artifact of slow clearance rate, indicating feeding within a previous sampling interval, rather than feeding within the present sampling interval. To lend support to the interpretation of feeding periodicity data, and to establish the minimum sampling interval, gut clearance rate of *Baetis* was measured on June 27–28, 1990, in the circular stream system. Ninety-six *Baetis* were sampled from the East River and pooled at 06:00, a time of high gut fullness. Twelve animals were randomly selected immediately and processed to determine initial gut fullness. Groups of 12 animals were then transferred to scrubbed, food-free circular streams containing four scrubbed and boiled stones each. One chamber was sampled at each of the following intervals (time elapsed from 6:00): 0.5, 1, 2, 4, 8, 12, and 24 h. Stones were replaced every 4 h in the 8-, 12-, and 24-h chambers to minimize the possibility of algal growth on these stones, a phenomenon that has confounded previous attempts to measure gut clearance (Peckarsky et al. 1993). Gut fullness was measured fluorometrically and expressed as nanograms of pigment per *Baetis*.

Relationship of Gut Fullness to Mayfly Size

We used a narrow range of *Baetis* (see Table 3) in each of these experiments and homogenized larval size among treatments, recognizing that feeding periodicity of mayflies may be a function of larval size (Allan 1978b). Before macerating each *Baetis* individual for analysis of gut pigments, we measured head capsule width, determined sex, and computed dry weight using sex-specific regression equations (C.A. Cowan and C.R. Anderson, unpublished data). Last-instar individuals, which often had empty guts, and those parasitized by mermithid nematodes were not used in the analysis. For each feeding periodicity trial, we determined whether mayfly sizes differed between treatments (by two-sample *t*-tests) and tested for associations between larval size and gut fullness using Spearman rank correlation coefficients.

Reciprocal Transplant Experiment

To test the hypothesis that the water in the East River, which contains fish cues, caused more strongly nocturnal diel periodicity in *Baetis*, reciprocal transplant experiments were conducted in June and August 1991. In this study, the microhabitat use of *Baetis* from each of the two streams was observed in the circular streams, using water from each stream. Thus, the periodicity of *Baetis* from the fishless stream was observed in both fishless stream water and in trout stream water, and similarly, trout stream *Baetis* were observed in water from both streams. Any differences in behavior could then be attributed to differences in water quality, one element of which is the presence or absence of fish cues. Experimental design was similar to that of the microhabitat use studies. Methodological constraints prohibited our conducting all four permutations simultaneously; thus, fishless stream *Baetis* were observed in June (on the overwintering generation) and trout stream *Baetis* were observed in August (on the summer generation). Five *Baetis* were placed in each of seven chambers and observed every 4 h for 24 h, except for the fishless stream *Baetis* in trout stream water, which were observed every 2 h during the day and every 4 h at night. Data were analyzed as described

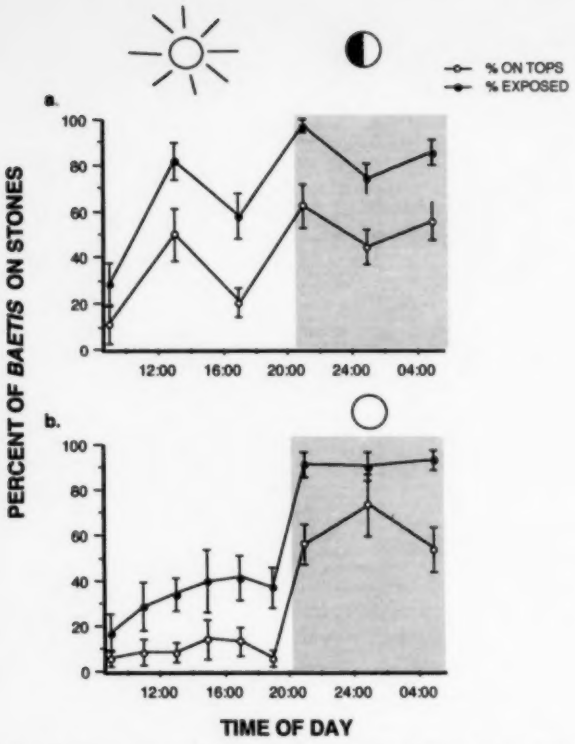

FIG. 2. Percentage of those *Baetis* on stones using stone tops (open circles) or exposed surfaces (tops + sides) (solid circles) over 24 h in the microhabitat use experiments conducted in circular streams. (a) Fishless stream water, June 1991; (b) trout stream water, June 1991. Values are means ± 1 SE. Shaded area indicates hours of darkness; moon phase is indicated above the shading.

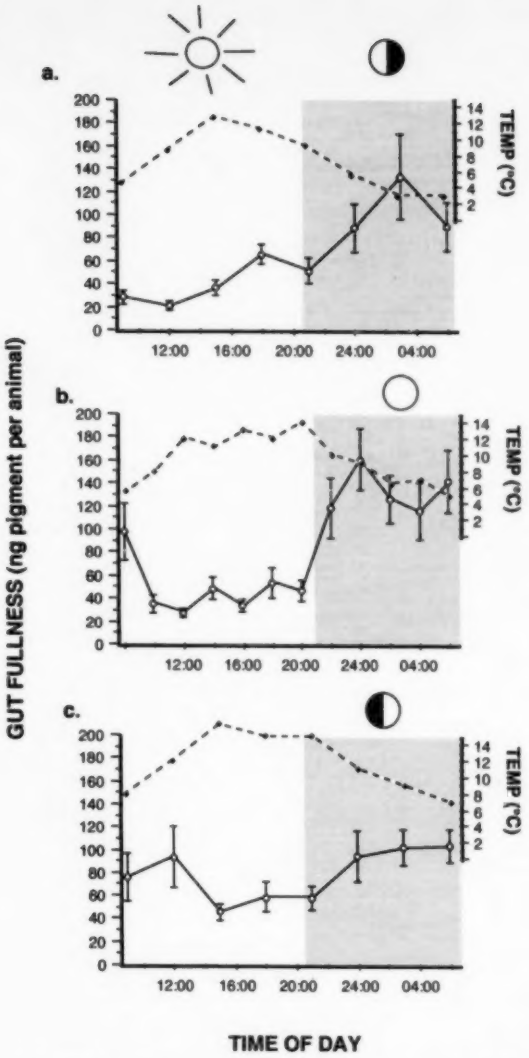

FIG. 3. Gut fullness (open circles) of *Baetis* over 24 h in the trout stream feeding periodicity studies. (a) June 1990; (b) July 1989; (c) August 1991. Stream water temperature at the time of sampling is indicated by the solid diamonds. Values are means ± 1 SE. Shaded area indicates hours of darkness; moon phase is indicated above the shading.

previously for the microhabitat use experiments. In addition, differences in periodicity across water types were compared for *Baetis* from the same source using two-sample *t*-tests. Thus, arc sine square-root transformed daytime values of trout stream *Baetis* in trout stream water were compared with daytime values of trout stream *Baetis* in fishless stream water, and likewise for nighttime values. The same analysis was applied to the study of fishless stream *Baetis*.

Results

Substrate Use Experiments

In the July 1985 and September 1987 observations, the percentage of *Baetis* using stone tops was significantly greater at night than during the day (Fig. 1a and 1c; Table 2). In August 1985, use of stone tops was uniformly low and aperiodic (Fig. 1b; Table 2). Thus, the winter cohort of *Baetis* in the fishless stream was nocturnal, while the summer cohort was aperiodic; summer cohort *Baetis* from the trout stream were also nocturnal. Correlations between temperature and percentage of *Baetis* on stone tops were not significant in July 1985 (Spearman's $r = -0.314$; $p = 0.544$), August 1985 ($r = 0.029$; $p = 0.957$), or September 1987 ($r = 0.0857$; $p = 0.872$).

Microhabitat Use Experiments

In circular chambers with fishless stream water, *Baetis* exhibited a weakly nocturnal peak in the use of stone tops (Fig. 2a; Table 2). A similar trend was observed for the percentage of animals using exposed (tops + sides) surfaces. Percentage on tops was lowest at midmorning (09:00) and greatest just after sunset (21:00). Use of stone tops was conspicuously heavy during the afternoon (13:00), at which time >50% of *Baetis* on stones were on the tops. In contrast, *Baetis* from the trout stream exhibited a very distinct nocturnal peak in use of stone tops and exposed surfaces (Fig. 2b). The proportion using stone tops was consistently

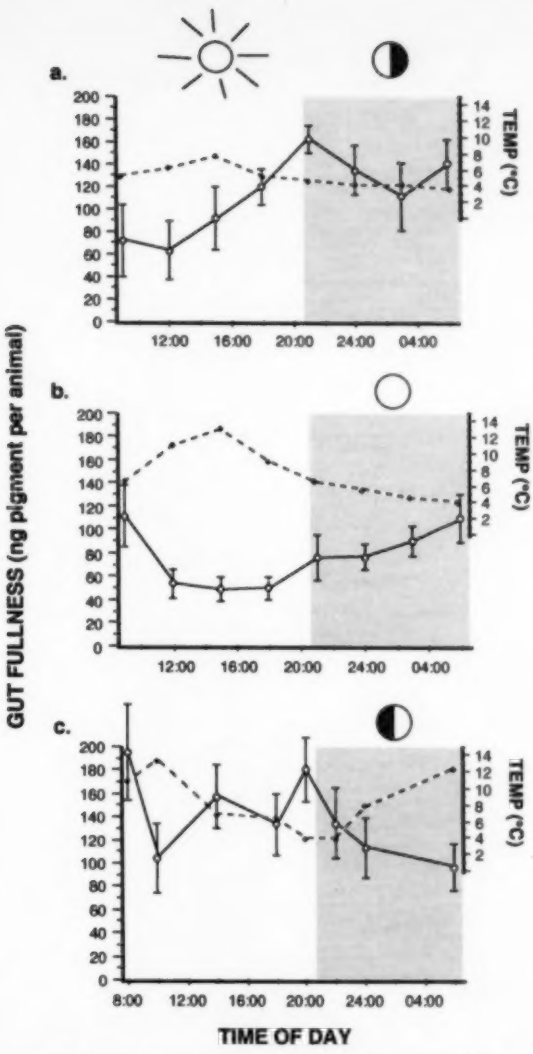

FIG. 4. Gut fullness (open circles) of *Baetis* over 24 h in the fishless stream feeding periodicity studies. (a) June 1990; (b) July 1990; (c) August 1989. Stream water temperature at the time of sampling is indicated by the solid diamonds. Values are means ± 1 SE. Shaded area indicates hours of darkness; moon phase is indicated above the shading.

low during the day (<15%) and increased just after sunset, remaining high (>50%) throughout the night. The proportion of *Baetis* using stone tops during the day was significantly lower in the trout stream than in the fishless stream (Wilcoxon test, $z = 2.179$; $p = 0.029$), while nighttime values did not differ ($z = 1.023$; $p = 0.306$) (Fig. 2). Percentage of *Baetis* on stone tops was not significantly correlated with temperature in either the fishless stream (Spearman's $r = -0.086$; $p = 0.872$) or the trout stream in June ($r = -0.160$; $p = 0.682$).

Feeding Periodicity

Baetis gut fullness was greatest at night in both the trout stream (Fig. 3) and the fishless stream (Fig. 4), with the single exception of the August 1989 fishless stream study, in which *Baetis* feeding was aperiodic. However, the magnitude of difference between night and day gut fullness was much greater in the trout stream than in the fishless stream, due to lower daytime values in the trout stream (Table 2); nighttime gut fullness in the two streams was similar. Interestingly, feeding periodicity in both streams varied seasonally. Fishless stream *Baetis* shifted from strongly nocturnal in June (Fig. 4a) to weakly so in July (Fig. 4b) and aperiodic in August (Fig. 4c). Winter cohort trout stream *Baetis* were strongly nocturnal, with very low daytime feeding rates, on both the June and July sampling dates (Fig. 3a, and 3b), while summer cohort *Baetis* were only weakly nocturnal (Fig. 3c).

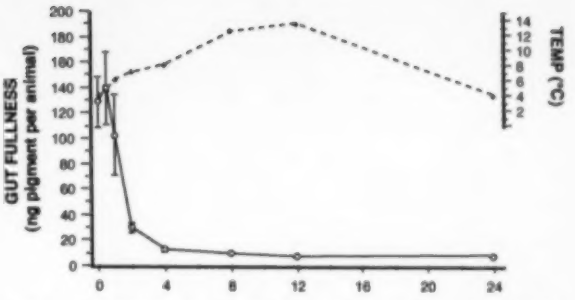

FIG. 5. Gut clearance of *Baetis* over 24 h (open circles). Water temperature in the stream chambers at the time of sampling is indicated by the solid diamonds. Values are means ± 1 SE.

In contrast with periodicity of substrate and microhabitat use, feeding periodicity was frequently significantly correlated with temperature. Negative correlations were obtained between temperature and gut fullness in the fishless stream in June 1990 (Spearman's $r = -0.687$; $p = 0.059$) and July 1990 ($r = -0.790$; $p = 0.019$) and in the trout stream in July 1989 ($r = -0.655$; $p = 0.021$) and August 1991 ($r = -0.850$; $p = 0.007$) (Fig. 3b, 3c, 4a, and 4b). Correlations between temperature and gut fullness were nonsignificant in the fishless stream in August 1989 ($r = 0.215$; $p = 0.608$) (Fig. 4c) and in the trout stream in June 1990 ($r = -0.515$; $p = 0.191$) (Fig. 3a).

Gut Clearance Time

Pigments disappeared from guts in 2–4 h (Fig. 5), indicating that the sampling resolution in the feeding studies was realistic: guts could have cleared within the sampling interval if feeding had ceased. Because this study was conducted at the coldest time of day (water temperature at beginning of experiment = 6°C), and because *Baetis* were not feeding during the experiment, this measure of gut clearance time is at the lower end of the range of possible values. At higher temperatures (Kiørboe et al. 1982; Dam and Peterson 1988), and when animals are feeding (Kiørboe and Tiselius 1987; Christofferson 1988), guts should clear more rapidly. With this conservative estimate of gut evacuation time, we can conclude that not only gut fullness, but feeding rate as well, was greater at night than during the day.

Relationship of Gut Fullness to Mayfly Size

For all feeding periodicity trials, either sizes of *Baetis* used in experiments did not differ between treatments or

TABLE 3. Summary of mean (±SE) dry weight (mg) and number of female and male *Baetis* larvae used in day and night treatments for each feeding periodicity trial, *t*-tests for differences in mean larval sizes between the treatments, and correlation coefficients between mayfly size and gut fullness.

		Day			Night						
Treatment	Date	Weight	♀	♂	Weight	♀	♂	t	$p > t$	R	$p > t$
No fish	June 1990	0.54 ± 0.02	27	21	0.68 ± 0.03	37	11	−3.80	0.0002	0.16	ns
No fish	July 1990	0.45 ± 0.01	34	6	0.46 ± 0.02	30	10	−0.43	ns	0.07	ns
No fish	Aug. 1989	0.68 ± 0.02	38	12	0.65 ± 0.04	20	10	0.76	ns	0.26	0.02
Fish	June 1990	0.63 ± 0.03	24	24	0.59 ± 0.03	24	7	0.90	ns	0.05	ns
Fish	July 1989	No size data									
Fish	Aug. 1991	0.47 ± 0.01	19	21	0.49 ± 0.15	23	17	−0.94	ns	−0.02	ns

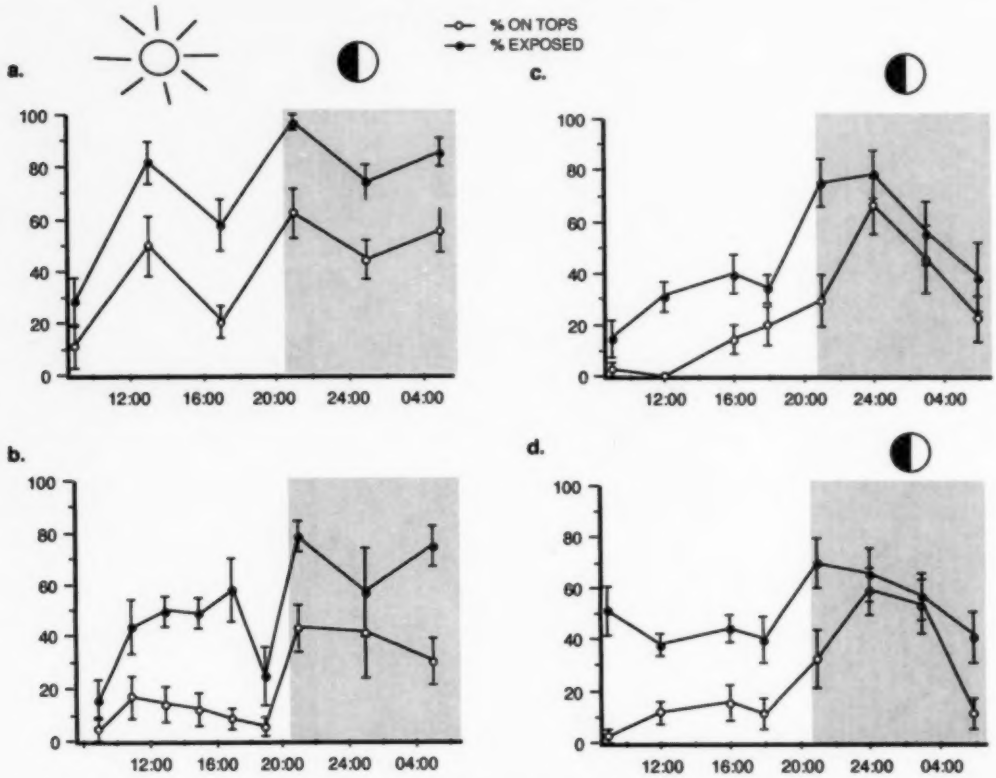

FIG. 6. Percentage of those *Baetis* on stones using stone tops (open circles) or exposed surfaces (tops + sides) (solid circles) over 24 h in the reciprocal transplant experiment. (a) Fishless stream *Baetis* in fishless stream water; (b) fishless stream *Baetis* in trout stream water; (c) trout stream *Baetis* in trout stream water; (d) trout stream *Baetis* in fishless stream water. Values are means ± 1 SE. Shaded area indicates hours of darkness; moon phase is indicated above the shading.

there were no correlations between mayfly size and nanograms of pigment per gut (Table 3). In most cases, both conditions were met. Therefore, treatment differences in gut fullness could not be attributed to size-dependent differences in mayfly feeding rates.

Reciprocal Transplant Experiment

In transplant experiments, fishless stream *Baetis* larvae were nocturnal when observed in fishless stream water (t = 4.10; p = 0.006) (Fig. 6a), and also when observed in trout stream water (t = 4.60; p = 0.004) (Fig. 6b). However, the percentage of *Baetis* using stone tops during the day was greater in fishless stream water (mean ± 1 SE = 27.4 ± 5.6%) than in water from the trout stream (12.7 ± 3.0%) (two-sample t-test: t = 2.25; p = 0.044). Nighttime stone use in fishless water was similar to that in fish water (t = 1.52; p = 0.171). These results suggest that use of stone tops during the day decreased when fish cues were present, yet microhabitat use during darkness was unaffected.

Trout stream *Baetis* were distinctly nocturnal in water from both the trout stream (t = 7.47; p = 0.0002) (Fig. 6c) and the fishless stream (t = 7.43; p = 0.0003) (Fig. 6d). However, in contrast with fishless stream *Baetis*, there were

no differences in use of stone tops between *Baetis* in the two water sources during the daytime ($t = 0.024$; $p = 0.981$) or the nighttime ($t = -0.34$; $p = 0.708$). Thus, it appears that removing fish cues had no effect on periodicity over 24 h, while the addition of fish cues resulted in a significant reduction in daytime use of stone tops.

Discussion

Expression of diel periodicity in feeding or habitat use is often a response to environmental or biological factors that impose physical constraints or increased risk of foraging during some periods. Several factors may account for nocturnal periodicity in *Baetis*. Kovalak (1978) suggested that changes in dissolved oxygen content of stream water may drive animals out of interstices to stone tops, where oxygen availability is greater (Wiley and Kohler 1980). Allan et al. (1991) monitored dissolved oxygen during periodicity studies in Cement Creek, a nearby high-elevation trout stream similar to the East River. They found that dissolved oxygen levels were actually highest at night and were overall very high (7–9 mg/L) and probably not limiting at any time of day. Daytime dissolved oxygen was similarly high in the East River (7.3–9.4 mg/L; 89–106% saturation (B. Barr and S. Tavormina, Rocky Mountain Biological Laboratory, unpublished data)) and was saturated as well in Benthette Brook (L.A. Martinez and B.L. Peckarsky, unpublished data). It is therefore improbable that dissolved oxygen is responsible for the nocturnal pattern of periodicity observed in this study.

Allan et al. (1986) found positive correlations between activity of *Baetis* on stone tops and stream temperature and reasoned that water temperature may account for diurnal activity of *Baetis* nymphs. In our study, use of upper stone surfaces (which does not indicate activity level) was not correlated with water temperature in either the substrate use or microhabitat studies. Thus, the presence or absence of *Baetis* on stone tops was not a function of stream temperature. In contrast, gut fullness of *Baetis* was frequently significantly correlated with water temperature. That the correlations were negative rules out the possibility that increased gut fullness (and therefore feeding rate) is a simple function of increasing temperature. It is more probable that stream temperature is correlated with a second factor, daylight, which is directly driving *Baetis* periodicity. Examination of the feeding periodicity graphs supports this hypothesis; sharp increases and decreases in gut fullness correspond directly with the light/dark cycle, while temperature lags behind. We conclude that temperature does not account for nocturnal periodicity in *Baetis*, nor does it serve as a proximal cue for periodic changes in behavior.

A biological factor potentially influencing diel activity is invertebrate predation. Two stonefly species, *Kogotus modestus*, and *Megarcys signata* (Plecoptera: Perlodidae), are abundant in the East River and Benthette Brook, and both species feed selectively on *Baetis* (Peckarsky and Penton 1989). These predators are crepuscular, active, and feeding in the early morning (*Kogotus*), late afternoon (*Megarcys*), and throughout the night (both species) (L.A. Martinez, University of Southern Colorado, unpublished data). If *Baetis* periodicity was an avoidance response to stonefly predators, the predicted pattern of periodicity would be diurnal rather than nocturnal, as we observed. Therefore, we can also rule out invertebrate predators as potential causal factors of *Baetis* nocturnal periodicity.

Another potential explanation of nocturnal periodicity in *Baetis* is avoidance of fish predation. Trout in the East River feed primarily during the day, and *Baetis* nymphs are an important item in their diets (Allan 1978a, 1981; Angradi and Griffith 1990). We hypothesize, as suggested by Allan (1978b), that the risk of trout predation imposes a constraint on *Baetis* foraging behavior by rendering stone tops unsafe during the day and that it is the primary factor shaping *Baetis* periodicity in our study system. We further suggest that the proximal cue for *Baetis* periodicity is the diel light cycle, which closely mirrors patterns of trout feeding.

Our results lend support to the fish predation hypothesis. In all studies, periodicity of *Baetis* feeding and substrate use was much more nocturnal in the East River than it was in the fishless tributary, Benthette Brook. Nighttime gut fullness levels were similar in the two streams, yet daytime values in Benthette Brook were at least two times those in the East River, indicating that larvae in the trout stream were eating much less during the day than their counterparts in the fishless tributary. In microhabitat use experiments, the night/day difference was again more dramatic in the East River. The proportion of *Baetis* using stone tops during the day in the June studies was much lower in the trout stream than in the fishless stream, while nighttime values did not differ. Similarly, in substrate use experiments, East River *Baetis* were distinctly nocturnal (Fig. 1c), while Benthette Brook *Baetis* were aperiodic (Fig. 1b) or more weakly nocturnal (Fig. 1a). We attribute these differences to the lack of fish in Benthette Brook and suggest that fish cues in the East River intensified the pattern of nocturnal periodicity.

Additional evidence for the influence of fish cues on periodicity is provided by the reciprocal transplant studies. When *Baetis* from the fishless stream were transplanted to water from the trout stream, their periodicity was much more marked, principally due to a reduction in the daytime use of stone tops by larvae in the fish water. Nighttime use of stone tops did not differ between the two water types, supporting our contention that fish presence imposes predation risk primarily during the day, when visual feeding occurs.

A notable result is that while fishless stream *Baetis* responded within 24 h to the addition of fish cues, the removal of fish cues did not change the periodicity of larvae from the trout stream over the short period of this study. Other workers have reported variable responses by organisms to risk addition and removal. For example, Palumbi (1984) observed that marine demosponge (*Halichondria panicea*) individuals from wave-swept (high stress) and calm (low stress) areas display different morphological types adapted to those habitats, the former being resistant to breakage, yet costlier to maintain. When genetically identical individuals were transplanted from one habitat to the other, new growth on sponges moved into high-stress areas quickly adopted the stress-resistant morphotype, while those transplanted into calmer waters took at least twice as long to adopt the faster growing, nonresistant morphotype. Palumbi (1984) concluded that acclimation to wave intolerance was delayed in transplants into benign environments because in an unpredictable environment the cost of acclamatory errors is great (41% tissue loss on average). We suggest that a

similar mechanism is driving the lack of response (or delayed response) of *Baetis* nymphs to the sudden removal of fish cues from the environment, in that the risk of responding to a "false alarm" outweighs the benefits of higher feeding rates during the day. This result is consistent with the observation that in stream environments, predator addition generally elicits faster population responses than does predator removal (Walde and Davies 1984; Cooper 1988).

We offer several mechanisms by which fish predation would render stone tops unsafe positions for *Baetis*. Drift entry, whether an active component of *Baetis* foraging behavior (Kohler 1985) or the result of passive erosion from an exposed position (Elliott 1967), is indisputably a "risky" behavior for *Baetis* in a trout stream during the day. Feeding may be restricted to nighttime because foraging and being on stone tops (and to a lesser extent, the sides) during the day would increase the probability of drift. In addition, although perhaps less importantly, there is evidence that fish, particularly rainbow trout (Angradi and Griffith 1990), and brook trout when sympatric with cutthroat trout (Griffith 1974) feed on the benthos as well as the drift (Tippets and Moyle 1978). Insects such as Trichoptera with low abundance in the drift are often overrepresented in trout stomachs (Allan 1978; Tippets and Moyle 1978) and are positively correlated with the occurrence of algae in fish guts (Tippets and Moyle 1978; Angradi and Griffith 1990), suggesting that they are picked out of the benthos. This fish feeding behavior could contribute as well to the diel migration of *Baetis* from stone tops at night to the bottoms during the day.

There are numerous examples of predators influencing the periodicity of aquatic invertebrate prey species. Flecker (1992) demonstrated not only that fish presence resulted in nocturnal drift by *Baetis*, but that as fish predation pressure intensified, the night to day drift ratio increased. Rader and Ward (1990) invoked fish predation risk to explain differences in *Baetis* periodicity above (nocturnal) and below (aperiodic) an impoundment. They concluded that increased filamentous algal cover (and refuge from fish predation) below the dam accounted for aperiodic use of stone tops by *Baetis*. Soderstrom (1989) found that the periodicity of the mayfly *Parameletus* varied within a population based on the presence or absence of fish in a river with fish versus a seasonally fishless tributary. Tjossem (1990) demonstrated experimentally that in the presence of fish cues, larval *Chaoborus*, the phantom midge, exhibited an increased intensity of diel vertical migration away from zones of fish feeding. Interesting parallels can also be observed in the crayfish *Orconectes propinquis*. Stein and Magnuson (1976) found that size classes of *Orconectes propinquis* (juveniles and females) vulnerable to smallmouth bass (*Micropterus dolomieu*, previously *Micropterus dolomieui*) predation exhibited nocturnal periodicity; adult males, too large for bass, were aperiodic. These examples, across a broad taxonomic array, indicate the precedent for phenotypic plasticity in prey periodicity as a response to the threat of predation. Further, we suggest that this phenotypic plasticity in *Baetis* feeding or positioning may account for much of the lack of agreement in the literature.

Finally, our results suggest in part that *Baetis* periodicity is also seasonally flexible. In general, winter generation *Baetis* were nocturnal (with one exception; Fig. 1c), while summer cohort *Baetis* were aperiodic or very weakly nocturnal (Tables 1 and 2), particularly towards summer's end (Fig. 3c and 4c). Other studies have demonstrated similar patterns. Kohler (1983) observed that in *Baetis* of the Pigeon River, Michigan, the incidence of "risky" behavior (daytime drift) increased in the summer cohort as it approached the season's end. He concluded that the summer cohort may trade off increased predation risk for faster growth rates (and therefore successful emergence and mating) achieved by continuous, unconstrained foraging. Rader and Ward (1990) found that small *Baetis* (0.01–0.10 mg), less vulnerable to fish predation (Allan 1978b), used stone tops more during the day than did larger *Baetis* (0.45–1.0 mg). The *Baetis* used in our study were of the larger of these two size ranges. When smaller nymphs were used (Fig. 4b), the pattern of nocturnal feeding was only very weak.

In conclusion, *Baetis* periodicity was variable according to stream type (fish/fishless) and season. Thus, we contend that contradictory reports of *Baetis* periodicity are not a phenomenon to be homogenized, resulting in the extraction of a single, "correct" answer to the question of *Baetis* periodicity, but rather one to be examined in greater detail. Factors such as fish presence/absence, fish predation intensity, and grazer size or seasonality may underlie apparent inconsistencies and generate interesting and testable hypotheses for future study.

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Return of Pacific Sardine (*Sardinops sagax*) to the British Columbia Coast in 1992

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The Pacific sardine (pilchard) (*Sardinops sagax*) supported an important commercial fishery in British Columbia for more than 20 yr before it suddenly collapsed in the mid-1940s. We report here the first confirmed catches of Pacific sardine in British Columbia since the 1950s and discuss the most likely explanation and possible future significance to British Columbia fisheries of the return of this species.

La sardine du Pacifique (*Sardinops sagax*) a soutenu une importante pêcherie commerciale en Colombie-Britannique pendant plus de 20 ans avant son effondrement brutal au milieu des années 40. Nous faisons ici état des premières prises confirmées de sardine du Pacifique en Colombie-Britannique depuis les années 50 et tentons d'expliquer les raisons les plus vraisemblables du retour de cette espèce ainsi que la signification possible de cet événement pour l'avenir.

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The Pacific sardine (*Sardinops sagax*), traditionally known as pilchard in British Columbia, supported the largest commercial fishery in the Western Hemisphere during the first half of this century (Wolf 1992). The spectacular decline of the sardine in the 1940s in both the eastern and western Pacific Ocean is well documented and has resulted in much debate about possible causes (e.g., Clark and Marr 1955; Murphy 1966; Ahlstrom and Radovich 1970). Historically, the northern sardine stock ranged from southern California to southeast Alaska (Hart 1973). This stock supported an important commercial fishery in British Columbia (B.C.) for more than 20 yr before it failed without warning in 1946 (Radovich 1982).

We report here the first confirmed catches of Pacific sardine in B.C. waters since the 1950s, discuss the most likely explanation for the reoccurrence of sardine in B.C. waters, and speculate on the possible future significance to B.C. fisheries.

Methods and Results

During June–October 1992, we conducted three surveys along the west coast of Vancouver Island (WCVI) using the Canadian Department of Fisheries and Oceans (DFO) research vessel *W.E. Ricker*, a 1100 gross tonne, 58-m (overall length) stern trawler. Each survey was conducted for a different purpose using different fishing gear. In each case the catches of sardine were incidental to the main targeted fish species.

The first survey (15 June – 4 July 1992) was conducted to assess the abundance and distribution of juvenile salmon (*Oncorhynchus*) along the WCVI. The sampling gear used was the Bernard–Sigmund beam trawl, a new paired-trawl gear designed to sample juvenile salmon and other small pelagic fishes between the sea surface and 15 m depth. During this survey, a total of 480 trawl tows were completed, in a rectangular grid pattern between 0.5 and 83 km offshore, starting at Cape Scott and ending at Cape Flattery (Fig. 1). A total of five Pacific sardine were caught in four separate, consecutive tows between 22:30 and 00:40 on 1–2 July off Barkley Sound (Fig. 1). These sardine were immediately measured (fork lengths 291, 273, 320, 272, and 285 mm) and then frozen at −40°C. Our identification of these fish as Pacific sardine was subsequently confirmed by Dr. Alex Peden, Head of Vertebrate Zoology and Curator of Fish, Royal British Columbia Museum, Victoria, B.C., who compared them with the reference collection (museum catalogue Nos. 930-2, 941-2, and 900-1) of Pacific sardine caught historically in B.C. waters.

The second survey was a midwater trawl survey designed to assess the abundance and distribution of Pacific hake (*Merluccius productus*) and other pelagic fish species off the southern WCVI. This survey was conducted between 29 July and 7 August 1992 and covered an area of 6000 km^2 of the continental shelf between Cape Flattery and 49°N latitude, from shore seawards to the 200-m isobath. The net used was a Diamond-7 midwater trawl, with a mouth opening of 20 × 13 m and a 4 × 4 cm stretched mesh liner in the codend. A total of 32 tows were completed, and 65 sardine were captured in five tows made between 4 and 6 August off Barkley Sound (Fig. 1). The largest catch of sardine (42 fish) was obtained in a set targeted on chub mackerel (*Scomber japonicus*) which were concentrated at 40–70 m depth. The lengths of 59 sardine and weights of 43 sardine which were in sufficiently good condition were determined immediately. The mean fork length and weight of sardine captured in all sets were 249.0 mm and 204.0 g, respectively. The resulting weight–length relationship (weight (g) = 0.000122·fork length (mm)$^{2.593}$) was highly significant ($n = 61$, $r = 0.961$, $p < 0.001$).

The third survey was conducted during 21 September – 2 October 1992 along the southern WCVI, in the same gen-

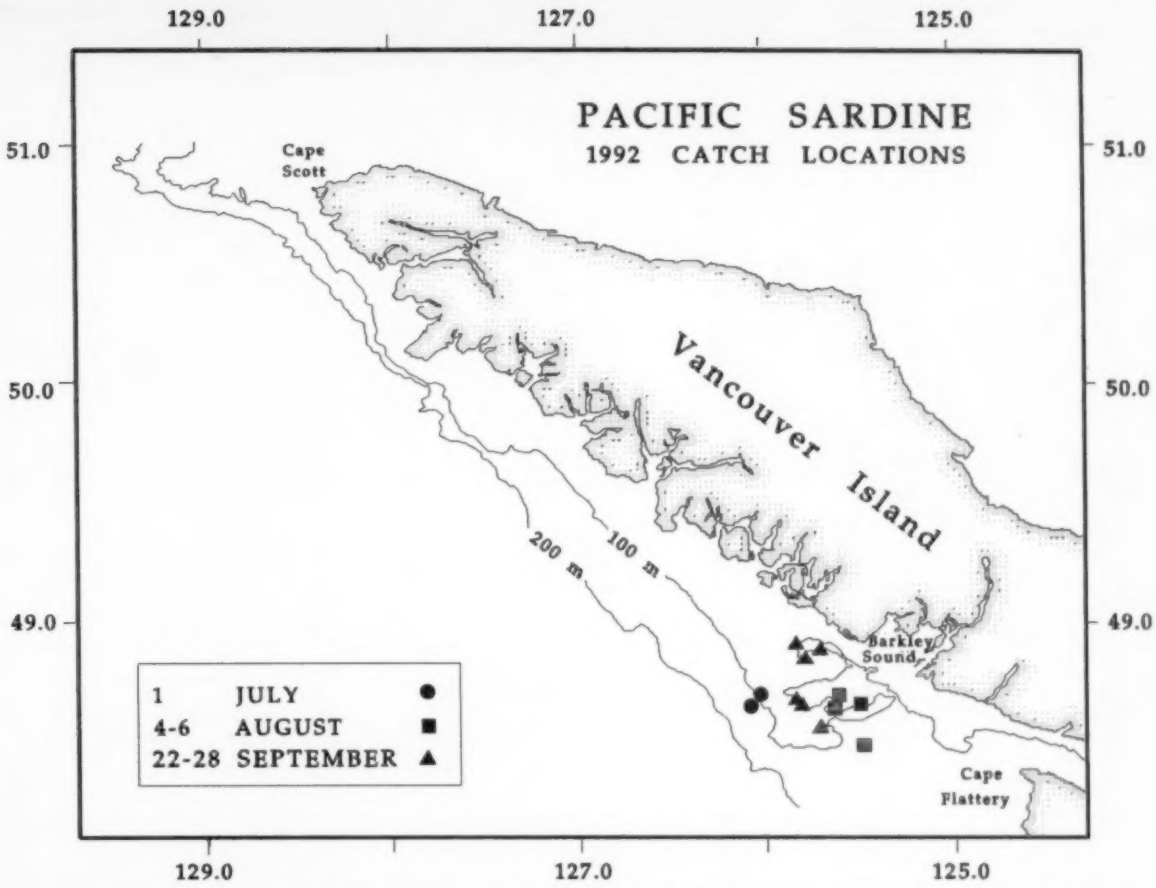

FIG. 1. Locations where one (cross-hatched symbols) or more (solid symbols) Pacific sardine were caught in B.C. waters on three research surveys during July–September 1992.

eral area as the second survey. The purpose was to determine the distribution and abundance of juvenile Pacific cod (*Gadus macrocephalus*) and other groundfish species. The fishing gear used was a Yankee-36 bottom trawl, fished with 900-kg steel doors. A total of 29 net tows were completed, and 31 sardine were caught in nine separate tows (average 3.4 sardine per tow) between 22 and 28 September 1992. These sardine were caught in two areas, one directly offshore of Barkley Sound and the other closer to shore and slightly further north (Fig. 1). The mean fork length and weight of the 23 sardine which were measured were 252.0 mm and 240.0 g, respectively.

The ages of 83 sardine caught on the August and September surveys were determined by examining their scales and otoliths. Two of these sardine were 1 yr old, 47 were between 2 and 3 yr old, 33 were between 4 and 7 yr old, and one sardine was 8 yr old. Determination of sardine ages using scales and burnt otolith surfaces gave similar results (within ±1 yr) for all but six fish.

Discussion

In the eastern North Pacific Ocean the Pacific sardine historically supported important commercial fisheries from California to B.C. The sardine fishery was conducted during the fall and winter months in California and during the summer in B.C. The main spawning area of the northern sardine stock was about 100 km south of the centre of the southern California Bight (Clark and Marr 1955). No spawning of Pacific sardine has been reported in B.C. (Hart 1973).

The northern stock of sardine undertook extensive annual feeding migrations northwards in early summer as far north as B.C. and occasionally southeastern Alaska (Marr 1960), returning to the spawning grounds each autumn. The northern extent of this annual migration increased each year as a sardine aged and grew larger.

The sardine fishery began in B.C. in 1917. Initially the catches were small, and most sardine were canned. In 1925, fishing regulations were amended to permit a sardine reduction fishery in B.C. waters, which led to rapid expansion of the fishery. Sardine landings in B.C. reached a peak of 80 558 t in 1943. The fishery failed unexpectedly in 1946 as the northern stock entered the final stages of its collapse (Radovich 1982), probably due to a combination of unfavourable environmental conditions and overfishing (MacCall 1979).

The Pacific sardine we caught off the WCVI in 1992 are the first confirmed sightings in B.C. waters since the collapse

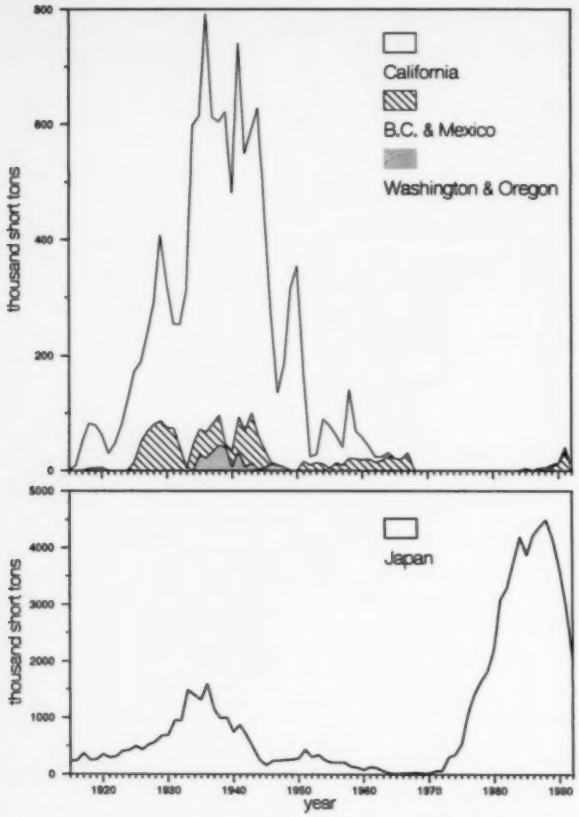

FIG. 2. Landings of Pacific sardine in the eastern Pacific (upper panel) and western Pacific Ocean (lower panel) (1 short ton = 0.9078 metric ton). Data from Wolf (1992), P. Wolf (California Department of Fish and Game, 330 Golden Shore, Suite 50, Long Beach, CA 90802, personal communication), Kuroda (1991), and T. Wada (Hokkaido National Fishery Research Institute, 116 Katsurakai, Kushiro, 085 Japan, personal communication).

of the sardine fishery in the late 1940s. No other sardine have previously been caught in extensive research surveys conducted annually by the DFO along the B.C. coast. To our knowledge, no sardine have been caught in the commercial, sport, or native fisheries since at least the mid-1950s. No confirmed catches of sardine in B.C. waters have been reported to the Provincial Museum since 1947 (A. Peden, Royal British Columbia Museum, Victoria, B.C., personal communication). We are aware of only one other report of sardine in B.C. waters since the 1950s. Fulton and LeBrasseur (1985) included an appendix which summarized unusual sightings of fish off the B.C. coast associated with the 1982–83 El Niño, including one report of Pacific sardine "schooling with anchovy in Clayoquot Sound" (located 60 km north of Barkley Sound along WCVI) in August 1983. However, we were not able to obtain any additional information to confirm this sighting.

The small number of sardine we caught along the southern WCVI probably does not reflect their true abundance in Canadian waters in 1992. We caught sardine on all three fishing surveys off the WCVI during a 3-mo period, indicating that sardine were present in this area during their historical mid-June to mid-October feeding period in B.C. waters (Hart 1943). However, each of these surveys was conducted for a different purpose, using fishing gear and sampling strategies targeted at other fish species. Sardine have traditionally been fished using purse seines rather than trawls. The sizes and ages of sardine we captured in 1992 indicate that these were mature fish and are comparable with sardine historically caught in Canadian waters. For example, Marr (1960) reported an average size (standard length) of sardine caught off B.C. of 243.0 mm (range 222.2 – 253.1 mm) for the period 1929–48 and an average age of 5.49 yr (range 3.66 – 7.50 yr) between 1934 and 1948.

What Caused Sardine to Reappear in B.C. Waters in 1992?

The return of sardine to B.C. waters in 1992 probably resulted from two coincident factors: (1) the current rapid rebuilding of the northern sardine stock off southern California and (2) a northward extension or shift in the range of the northern sardine stock due to the relatively strong 1991–92 El Niño (ENSO) oceanographic event. The northern sardine stock collapsed to low levels by the early 1960s, but began to rebuild after 1975 (Brewer and Smith 1982; Wolf and Smith 1986; Smith et al. 1992). As this stock recovers, the historical migration pattern will probably resume and eventually result in older sardine again entering B.C. waters each summer. However, sardine historically also tended to range farther south during cold winters and farther north during warm summers (Marr 1960; Radovich 1982). The relatively strong 1991–92 ENSO event in the North Pacific resulted in anomalous warm water conditions along much of the North American west coast. This probably assisted the penetration of sardine into Canadian waters, either by causing the older sardine to migrate farther north or by shifting the range of the entire population farther north in 1992.

Too Little, Too Late?

The future significance to B.C. fisheries of the reappearance of sardine in Canadian waters depends on how long the current rapid growth of the northern sardine stock is sustained. Statistically, the abundance of the northern sardine population in the eastern Pacific has tended to cycle over a period of about 60 yr during the past 1700 yr, with an average time of 36 yr required for sardine abundance to recover from cycle lows to cycle highs (Baumgartner et al. 1992). However, most sardine recoveries were also followed relatively quickly by a collapse. During this century the periods of high and low catches of the Asian, California, and Chilean sardine have been in phase, suggesting that a common process may control sardine abundance throughout the Pacific (Kawasaki and Kumagai 1984; Lluch-Belda et al. 1989). However, the California sardine population has not recovered as strongly as the Asian sardine in the most recent cycle (Fig. 2). Also, Japanese catches of sardine have decreased dramatically since 1989 (Fig. 2), possibly indicating the onset of another collapse of the Asian sardine. If there is a common process controlling the cyclic pattern of sardine production throughout the North Pacific, then the current rebuilding of sardine in the eastern Pacific may soon end. In other words, the recovery of the northern

California sardine stock may have occurred too slowly and too late to result in high abundance during this current production "cycle" of sardine in the North Pacific. In the first half of 1992 the catches of sardine off the southern California coast (Fig. 2) were lower than expected (Wolf 1992). Alternatively, if Pacific sardine production cycles are not completely synchronous over longer time scales, then the recent rebuilding of sardine in the eastern Pacific may continue, and signal the beginning of a new production cycle. This could soon result in the annual migration of sufficient numbers of sardine into northern waters to support an important "new" fishery in B.C. and also profoundly affect other species which currently dominate the pelagic environment of the eastern Pacific Ocean (Ware and Thomson 1992).

Note Added in Proof

In March 1993, two Pacific sardine were captured in a seine set made during the herring test fishery in the Lambert Channel and Baynes Sound area in the Strait of Georgia. Ron Tanasichuk (Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., personal communication) discovered both sardine and measured one: a 23-cm male (with developing gonads). Mr. Tanasichuk estimated that sardine accounted for an estimated 0.01% of the herring test fishery catch. During our annual midwater trawl survey off Barkley Sound in August 1993, Pacific sardine were caught in 22% of the tows, compared with 15% during the 1992 survey in the same area. In one tow, we captured 0.5 tonne of sardine. These observations highlight that (1) some sardine remained in British Columbia during the winter of 1992–93 and (2) sardine were more widely distributed, and possibly more abundant, off Barkley Sound in August 1993 than they were in 1992.

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Spatial and Temporal Variations in Chemical Contamination of American Eels, *Anguilla rostrata*, Captured in the Estuary of the St. Lawrence River

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Levels of polychlorinated biphenyls (PCBs), mirex, and pesticides were 10–100 times higher in migratory adult American eels, *Anguilla rostrata*, sampled at Kamouraska, Quebec, in 1990 than in eels from an uncontaminated reference tributary; concentrations in eels within the estuary varied little among sites. In contrast, mercury levels were the same at estuarine and reference sites, suggesting natural sources or atmospheric deposition of mercury. Dioxins, furans, and polynuclear aromatic hydrocarbons were virtually absent at all sites. During the 7 wk of migration, levels of PCBs, mirex, and pesticides in eels increased, while mercury did not. Since 1982, levels of PCBs and mirex have declined by 68 and 56%, respectively, and the percentage of eels exceeding human health guidelines for PCBs and mirex was about twofold lower in 1990 than in 1982. Levels of other pesticides have also declined, except that dieldrin is unchanged since 1982. While chemical concentrations are declining, levels of specific, highly toxic PCB congeners are sufficiently high that eel consumption by beluga whales (*Delphinapterus leucas*) is still likely to be hazardous. The hazard to the eels themselves is unknown due to a scarcity of toxicity data, but the highest concentrations of chemicals were observed in gonads.

Chez des anguilles d'Amérique, *Anguilla rostrata*, adultes en migration, échantillonnées à Kamouraska (Québec) en 1990, les concentrations de biphényles polychlorés (BPC), de mirex et de pesticides étaient 10 à 100 fois plus élevées que chez les anguilles provenant d'un affluent témoin non pollué; dans l'estuaire, les concentrations variaient peu chez les anguilles d'un site à l'autre. Par contre, les teneurs en mercure étaient les mêmes dans l'estuaire et aux sites témoins, ce qui semble indiquer l'existence de sources naturelles ou de dépôt d'origine atmosphérique du mercure. Les dioxines, les furanes et les hydrocarbures aromatiques polynucléaires étaient pratiquement absents de tous les sites. Pendant les sept semaines de la migration, les concentrations de BPC, de mirex et de pesticides chez les anguilles augmentaient, mais pas les teneurs en mercure. Depuis 1982, les concentrations de BPC et de mirex ont baissé respectivement de 68 à 56%, et le pourcentage d'anguilles chez lesquelles la teneur en BPC et en mirex dépassait le niveau autorisé pour la santé humaine était environ deux fois plus bas en 1990 qu'en 1982. Les concentrations d'autres pesticides ont aussi baissé, sauf celle de dieldrine qui ne bouge pas depuis 1982. Tandis que les concentrations de produits chimiques diminuent, les teneurs en certains congénères de BPC extrêmement toxiques restent assez élevées pour que la consommation d'anguilles par le béluga (*Delphinapterus leucas*) reste dangereuse. Le risque pour les anguilles elles-mêmes n'est pas connu, à cause de la rareté des données sur la toxicité, mais c'est dans les gonades qu'on a observé les concentrations de produits chimiques les plus élevées.

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American eels, *Anguilla rostrata*, spawn in the Sargasso Sea, migrate as larvae to coastal streams of North America, and spend most of their lives maturing in freshwater (Schmidt 1925). In the St. Lawrence drainage basin, eels can ascend as far as Lake Ontario and may spend 12–16 yr growing from a size of <1.0 g to 4000 g

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(Castonguay et al. 1994). Their diet ranges from macroinvertebrates to small fish, and they accumulate high concentrations of the chemical contaminants commonly found in the Great Lakes ecosystem (Castonguay et al. 1989).

The accumulation of contaminants by eels may cause toxicity both to the eels and to their predators. Mass mortalities of eels have occurred in the freshwater portions of the St. Lawrence River, deformities have been noted by fishermen in the estuary, and the number of juveniles migrating towards Lake Ontario has diminished by more than 98% since 1985 (Homer 1986; Dutil et al. 1987; Hendrick 1991). Mass mortalities were associated with gill damage and impaired ionoregulation, likely due to the combined effects of osmotic stress from saltwater adaptation and gill damage due to migration through industrial effluents (Dutil et al. 1987). Chronic toxicity was not suspected, since these effects did not correlate with tissue levels of persistent chemicals such as polychlorinated biphenyls (PCBs). However, the analyses did not measure highly toxic compounds now known to occur in the aquatic environment, such as specific congeners of PCBs, polychlorinated dibenzo[*p*]dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) (Eisler 1986).

Recent studies of beluga whales (*Delphinapterus leucas*) in the estuary and the Gulf of St. Lawrence suggest that contamination of eels may have other important effects. Dead beluga contain levels of PCBs, DDT, pesticides, and mirex far above those found in beluga from the Arctic (Massé et al. 1986; Martineau et al. 1987; Muir et al. 1990). Marine mammals are probably exposed via their diet, since there is no contact of respiratory surfaces with water, which in fish permits direct chemical uptake. Therefore, the majority of contaminants in beluga must come from prey consumed over their lifetime. Since beluga are long-lived (20–30 yr), are predatory, and do not grow after 6–10 yr of age, they should accumulate high levels of lipophilic, persistent compounds such as PCBs and mirex. However, concentrations measured in beluga are too high to be explained simply by the consumption of fish from the estuary (Béland et al. 1987; Hickie et al. 1991). Furthermore, mirex is not often found in estuarine fish, but was thought to be unique to fish from the upper St. Lawrence River and Lake Ontario, the only Great Lake to receive direct inputs (Kaiser 1978; Dutil et al. 1985; Sloterdijk 1985; Castonguay et al. 1989). Since eels contain contaminants at levels 10 times those of estuarine fish, and since a high proportion contain mirex due to their exposure in Lake Ontario, they may be an important transport route of chemicals to beluga (Lum et al. 1987; Béland and Martineau 1988; Hickie et al. 1991).

We collected and analyzed migratory adult eels ("silver eels") from the St. Lawrence estuary in 1990 to evaluate the effects of contaminants on their health, the role of eels in contaminating beluga whales, and the changes in contamination since the last major survey in 1982 (Desjardins et al. 1983a, 1983b; Dutil et al. 1985; Castonguay et al. 1989). Since eels migrate from many tributaries to the St. Lawrence, as well as from Lake Ontario, it cannot be assumed that a sample taken at one place or time represents all eels. Indeed, the freshwater origin of silver eels can be distinguished by their burden of mirex (Dutil et al. 1985; Castonguay et al. 1989), and deformed eels are more common at the end of the annual run than at the beginning (Homer 1986). Therefore, heterogeneity in a sample of eels and in their contaminant burden could be induced by the distance they must migrate (e.g., those starting in the Great Lakes versus those starting in tributaries closer to the estuary), their ability to swim (toxicity, deformities), and their river of origin and distribution in the estuary. Since the eel fishery is coastal and eels can be captured on both the north and south shores, it is possible that the eels migrate along a coast according to their river of origin.

Therefore, we tested the following null hypotheses: (a) levels of chemicals do not occur at concentrations that are toxicologically significant, (b) there are no temporal changes in average levels of chemicals in eels during the annual migration, as indicated by weekly sampling of migrating eels, (c) contamination has not changed since 1982, the last major sampling of migrating eels, and (d) contamination of migrating eels does not vary spatially, as shown by eels sampled within the St. Lawrence estuary and in a tributary to the estuary. Since eels from the St. Lawrence estuary were larger than those sampled in a tributary, size may confound comparisons among locations. Therefore we tested the hypothesis that (e) contamination of migrating eels does not vary with size, as measured by whole wet weights at the time of sampling. Furthermore, since beluga would eat whole eels, but only gutted carcasses are analyzed in surveillance programs, we compared the levels of chemicals in whole eels with levels in specific tissues, testing the hypothesis that (f) concentrations of contaminants do not vary among tissues or between tissues and "whole body".

Materials and Methods

This study focussed on maturing eels migrating seaward for spawning. While the term "silver eels" has been applied to this stage, colour was not a criterion for sampling, since colour is highly variable and can change within hours (Scott and Crossman 1973). The criterion used was simply capture in commercial or experimental traps oriented to intercept those eels migrating seaward. While size varied considerably, advanced maturity was confirmed by histological examination of oocytes (C.M. Couillard, unpublished data); all fish sampled were female.

Sample Collection

Random samples of about 100 live silver eels were purchased from a commercial fisherman at Kamouraska, Quebec (Fig. 1). Samples were collected weekly during the entire 7 wk of the annual fishery, between September and November 1990. During this 7-wk period, live eels were also purchased on one occasion each from fishermen at Cacouna, on the south shore of the estuary, and at Saint-Irénée, on the north shore. The eels were trapped during their seaward spawning migration by commercial weirs installed at angles to the shoreline. Most of these eels would be from the St. Lawrence River watershed, upstream of Quebec City (Dutil et al. 1985). Live eels were also captured by experimental trap nets during their seaward migration in Rivière aux Pins (Fig. 1), an uncontaminated reference stream that enters the sea from the north shore of the St. Lawrence estuary.

All eels were returned live to the laboratory where they were held in clean, flowing salt water (30 parts per thousand salinity) for 6–48 h before being killed for tissue analyses. Each was anaesthetized in MS-222 (tricaine

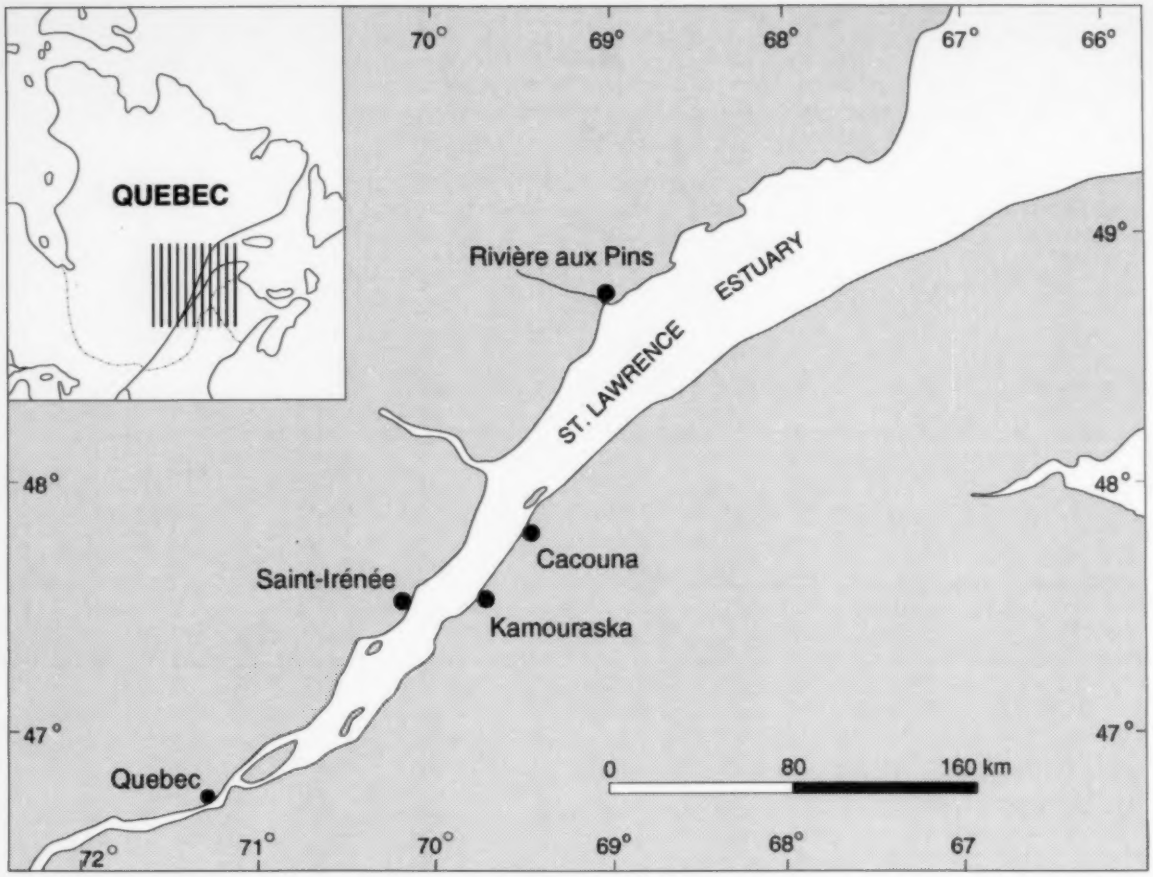

FIG. 1. Sampling sites. Eels were purchased at commercial weirs at Kamouraska, Saint-Irénée, and Cacouna in the estuary in salt water and were captured by a trap set in freshwater in Rivière aux Pins.

methanesulfonate), the viscera and gonads were removed, and the carcass with head attached was frozen in a contaminant-free plastic bag (ARCAN Inc., Plainswell, Mich.).

After all sampling was completed, the frozen eels from each week and each site were subsampled to provide two each with an original live weight of about 300, 600, 900, 1200, 1500, 1800, 2100, 2400, 2700, or 3000 g. The smallest and largest eels were scarce so that N varied from 7 to 16 fish per sample. For eight additional eels, analyses were made of the head, gutted carcass, gonad, and viscera (liver, stomach, intestines, etc.) to compare whole fish and tissue contaminant levels. For polynuclear aromatic hydrocarbons (PAH), PCDD, and PCDF analyses, only a limited number of fish were analyzed due to cost. Homogenates of two fish in the 900-g weight class were pooled to provide one sample for each of the seven sampling times at Kamouraska and one for the fish collected at Rivière aux Pins.

The fish from Kamouraska were purchased at the same location as in 1982, and chemical analyses were by the same laboratory with the same methods as in 1982, to maintain continuity of monitoring data (Desjardins et al. 1983a, 1983b).

Sample Preparation

Each eel was thawed, the head removed, and the remaining gutted carcass (muscle, skeleton, and skin) homogenized with a Hobart meat grinder using a 4-mm hole size. The homogenate was collected in stainless steel bowls, rehomogenized, and stored as 100-g aliquots in glass jars, using stainless steel or plastic kitchen utensils rinsed in ultrapure hexane. The glass jars were also rinsed in hexane, and a liner of hexane-rinsed foil separated the homogenate from the plastic jar lid. After each fish, the grinder was disassembled and cleaned, which included a hot water wash, scrubbing, rinsing with water, and rinsing with ultrapure hexane.

Individual tissue samples were homogenized in the Hobart grinder (head), or in a smaller laboratory grinder (viscera and gonads) to avoid excessive loss of material. It was not necessary to empty or clean the digestive tract, since eels do not feed during their migration and the stomach is partially resorbed.

All homogenates were frozen and maintained at −20°C until analysis.

Sample Analysis

Several chemicals were measured and detection limits varied according to the methods used. The methods are described in detail in Hodson et al. (1992) and summarized briefly here. Lipids were not measured in most samples, except for the analyses of individual tissues.

Carcasses

Organochlorine compounds were extracted from homogenates with an acetone–hexane (1:1) mixture and cleaned by gel permeation chromatography (GPC) and 2% deactivated Florisil (Johnson et al. 1976; Mills et al. 1972). Hexachlorobenzene (HCB), DDE, PCBs, and mirex (fraction F1) plus 12 other pesticides (fraction F2) (McLeod and Ritcey 1978) were measured by gas chromatography with an electron capture detector (GC-EC) (Sherma and Beroza 1980; Freeman 1981), using 2% OV-17 and 2.6% OV-210 packed columns. Three peaks were used to calculate total Aroclor 1254 (IUPAC Nos. 127, 147, and 177; Reynolds 1971). Recoveries ranged from 90 to 110% and were uncorrected. PCB congeners and pesticides were separated on a capillary column and quantified by peak-to-peak comparison (Martineau et al. 1987).

Mercury was analyzed in acid-digested samples by selective reduction to elemental mercury by $SnCl_2$, using a Technicon autoanalyzer with a mercury monitor detector (Armstrong and Uthe 1971; MPO 1989).

For analyses of PCDDs and PCDFs, homogenates were mixed with anhydrous Na_2SO_4 and radiolabelled surrogates and extracted several times with dichloromethane (DCM). The combined extracts were concentrated to 5.0 mL, diluted 50:50 with cyclohexane, and cleaned up by GPC after centrifugation to remove particulates. The extracts were solvent exchanged into isooctane, and recoveries ranged from 98 to 114%. The extracts were cleaned following Dow methodology (Lamparski et al. 1979), and final extracts were evaporated and taken up in 10 μL of isooctane with 250 pg [$^3H_{12}$] triphenylene·μL^{-1} as an internal standard.

PCDDs and PCDFs were measured with a VG70-VSE high-resolution gas chromatograph mass spectrometer (GC-MS), and the elution windows for each congener group were established by injecting a mixture containing the first and last congener to elute. Concentrations were estimated from responses of individual 2,3,7,8-substituted standards. Average responses for the 2,3,7,8- substituted congeners of each congener group were used to calibrate the instrument response for all isomers of that group. Individual congeners were identified by their specific ions and ion ratios at an instrument resolution of 1 in 10 000. Identification was also made with a minimum signal-to-noise ratio of 3 to 1 and a retention time within a 3-s window of external standards after internal standard correction. None of the data were blank corrected but they were corrected for surrogate recoveries.

For PAHs, tissue extracts were fractionated on a silica column. Fraction F2 was retained, reduced to 0.5 mL, and an internal standard added prior to GC analysis with a flame ionization detector (GC-FID) (Hodson et al. 1992). Compounds were identified by comparing retention times with authentic standards (Supelco PAH Kit 610-M; Supelco Canada Ltd., Oakville, Ont.). All peaks identified as PAHs by the retention time method were cross checked by GC-MS.

Tissue comparison

Fish samples (10–12 g wet wt) were mixed with anhydrous Na_2SO_4 and extracted three times with 50 mL of hexane–acetone (70:30) at 35°C. The extracts were reduced in volume, particulates were removed by centrifugation, and lipid was removed by GPC (Norstrom et al. 1986). Samples eluted in DCM–hexane (50:50) were evaporated to 0.5 mL and split into two fractions using a florisil column eluted by hexane (F1) and hexane–DCM (75:25) (F2). Fraction volumes were reduced to 0.5 mL and an internal standard was added for analysis.

Chlorinated compounds were analyzed by GC-EC by comparing retention times with those of a chlorinated pesticides standard (Supelpreme®–HC pesticides mix; Supelco Canada Ltd., Oakville, Ont.) or a PCB standard containing Aroclors 1242, 1254, and 1260 (1:1:1) (Supelco Canada Ltd., Oakville, Ont.). Congeners were identified according to Schultz et al. (1989). Identities of some major congeners were confirmed by GC-MS. The overall variability of the method was ≤15% and the detection limit 0.00001 μg·g wet wt^{-1}.

Total mercury was analyzed in duplicate by flameless atomic absorption spectrophotometry (AAS) against aqueous standards (detection limit = 0.005 μg·g wet wt^{-1}) after sample digestion at 55°C by nitric, sulphuric, and hydrochloric acids (after Hatch and Ott 1968). The overall variability of the method was ≤10%.

Total cadmium, copper, chromium, and zinc were determined in samples digested three times at 75°C in 50% hydrogen peroxide for 1 h, followed by concentrated HNO_3 for 2.5 h. Extracts were analyzed by graphite furnace AAS against aqueous standards. Wet weight detection limits were 0.01 μg·g^{-1} (Cd and Cr), 0.07 μg·g^{-1} (Cu), and 3 μg·g.$^{-1}$ (Zn) and variability was 4% for Cd, Zn, and Cu and 10% for Cr.

Toxicological Significance

The toxicological significance of PCB, PCDD, and PCDF congeners can be judged by equating their toxicity to that of the highly toxic 2,3,7,8-tetrachlorodibenzo[*p*]dioxin (TCDD). Based on similar modes of toxic action, the toxicity of each congener may be expressed as a fraction of the toxicity of TCDD (Safe 1990). When these toxic equivalent factors (TEFs) are multiplied by the concentration of the corresponding congeners, concentrations are expressed as TCDD equivalents (TEQs), which are summed over all congeners to give the total TEQs. Equivalents developed from mammalian models are used to estimate the risk to humans from consuming food contaminated by PCBs, PCDDs, and PCDFs and were used here to judge the hazard to beluga whales of consuming eels.

For other compounds, significance was judged by comparison of measured concentrations with Canadian Guidelines for Human Consumption for chemicals in fisheries products. Since the guidelines are intended to protect human consumers of fish, levels of chemicals exceeding the guidelines would presumably represent a risk to other mammalian consumers.

Statistics

All data except weights were converted to logarithms before statistical analysis due to the large ranges in values and the resultant nonnormal distributions and nonhomogenous variances. Log-transformed data met assumptions of normality and homogeneity of variance; contaminant levels are reported as geometric means. Many compounds were not detectable and a value of one half the detection limit was substituted for statistical analysis, a common convention in contaminant monitoring programs (D.M. Whittle, Department of Fisheries and Oceans, Burlington, Ont., personal communication).

TABLE 1. Ranges of geometric mean concentrations of eight PCB congeners, pesticides, mirex, and mercury in 89 eels sampled weekly for 7 wk at Kamouraska in 1990. The *F*-statistics are from ANOVA testing the effect of week of sampling, and *p* is the probability that variations among weeks were by chance. An asterisk indicates statistical significance at the 0.05 probability level.

Compound	Range of geometric mean concentrations ($\mu g \cdot g^{-1}$)	F_{weeks}	p
PCB congener			
28	0.002–0.012	2.98	0.011*
52	0.023–0.056	2.03	0.071
101	0.022–0.057	1.58	0.163
118	0.052–0.156	1.99	0.077
153	0.076–0.219	1.86	0.098
137	0.004–0.011	1.63	0.148
138	0.063–0.192	2.04	0.070
180	0.037–0.113	1.68	0.137
Sum of congeners	0.289–0.827	1.96	0.081
Total Arochlor 1254	0.612–2.133	2.04	0.069
Pesticides			
p,p'-DDE	0.058–0.280	2.14	0.058
p,p'-DDD	0.045–0.085	1.17	0.330
p,p'-DDT	0.019–0.071	1.43	0.216
Total DDT	0.125–0.458	1.81	0.107
Hexachlorobenzene	0.004–0.018	2.54	0.026*
α-BHC	0.005–0.007	1.65	0.143
Heptachlor	<0.001–0.001	2.26	0.046*
Heptachlor epoxide	0.008–0.021	2.68	0.020*
Lindane	0.002–0.003	1.28	0.275
Aldrin	<0.001–0.002	2.52	0.027*
Endrin	0.003–0.013	3.09	0.009*
Dieldrin	0.025–0.065	1.41	0.219
Oxychlordane	0.005–0.014	1.84	0.101
1-Chlordane	0.006–0.011	0.92	0.483
2-Chlordane	0.015–0.037	1.44	0.208
Total pesticides	0.229–0.701	1.78	0.114
Mirex	0.006–0.086	3.04	0.0099*
Mercury	0.18–0.26	0.93	0.48

For the study of tissue distribution, the "whole-body" concentrations of contaminants were calculated from the weights and chemical concentrations of the individual parts. For the eight eels tested, the carcass, viscera, gonads, and head averaged 83.3, 3.1, 4.9, and 8.7% of the whole-body wet weight. Hence, the concentration (C) of a chemical in the whole body was calculated as

$$C_{\text{whole body}} = (0.833 \times C_{\text{carcass}}) + (0.031 \times C_{\text{viscera}}) + (0.049 \times C_{\text{gonads}}) + (0.087 \times C_{\text{head}}).$$

Comparisons of chemical levels in carcasses among weeks were made by analyses of variance (ANOVAs) using the general linear model (GLM) available as a SAS computer routine (SAS Statistics for Applied Sciences, version 6.0, SAS Institute, Inc., Box 8000, Cary, NC 27512-8000). Site comparisons by ANOVA were made in pairs, since chemical levels varied with weeks. Hence, results at Rivière aux Pins, Cacouna, and Saint-Irénée were compared individually with results at Kamouraska for the corresponding weeks of sampling. The comparison of 1990 data with 1982 data was also made by ANOVA. All comparisons were made at the 95% confidence level, using Tukey's test to discriminate differences among means. To assess the effect of fish size on contamination, coefficients of determination (r^2) were calculated between chemical concentrations and total weight for all fish sampled at Kamouraska.

Results

Levels of Chemicals in Relation to Toxicity

Although only a small number of PCB congeners were measured in carcasses, they were found consistently and at concentrations well above the detection limit (Table 1). Congeners 28 and 137 (IUPAC nomenclature, Mullin et al. 1984) were the least concentrated and were found most frequently at levels less than 0.01 $\mu g \cdot g^{-1}$. Congeners 52 and 101 ranged from 0.004 to 0.336 $\mu g \cdot g^{-1}$ in individual fish, about one half to one third of the concentration of congener 180, which ranged from 0.010 to 0.524 $\mu g \cdot g^{-1}$. Congeners 118, 153, and 138 were most concentrated, with levels from 0.001 to 0.833 $\mu g \cdot g^{-1}$. The sum of the individual congeners ranged from 0.156 to 3.033 $\mu g \cdot g^{-1}$, while PCBs expressed as "Aroclor 1254", a specific mixture of congeners, ranged from 0.149 to 7.489 $\mu g \cdot g^{-1}$. However, these analyses were highly variable. Within any sample of 7–16 eels, concentrations could range from less than 0.2 $\mu g \cdot g^{-1}$ (reference site) to

TABLE 2. Summary of Canadian Guidelines for Human Consumption for the levels of chemicals in fishery products, and the percentage of fish that exceeded these guidelines at Kamouraska in 1982 and 1990 and at Rivière aux Pins in 1990.

	Guideline ($\mu g \cdot g^{-1}$)	Percentage of fish exceeding the guideline[a]			
		All eels, 1982	Kamouraska, 1982	Kamouraska, 1990	Rivière aux Pins, 1990
N		396	104	89	7
PCB (Arochlor 1254)	2.0	62	80	36	0
Mirex	0.1	31	52	29	0
2,3,7,8-TCDD	0.00002	—	—	0	0
DDT	5.0	0	0	0	0
Other pesticides	0.1	33.1	13.5	15.7[b]	0
Mercury	0.5	9.3	8.6	2.3	0

[a]Data for 1982 were derived from Desjardins et al. (1983a, 1983b), Castonguay et al. (1989), and C. Desjardins (unpublished data).
[b]Dieldrin in all cases.

greater than 2.0 $\mu g \cdot g^{-1}$. The coefficients of variation of geometric means for fish sampled weekly at Kamouraska ranged from 104 to 1058%, with a mean of 387%. For Aroclor 1254, 32 of 89 (36%) fish at Kamouraska exceeded the guideline for PCBs in fish products of 2.0 $\mu g \cdot g^{-1}$ (Table 2).

The analysis of PCB congeners in the eight fish used for the tissue comparison was much more detailed, and there was a wide variety in every tissue examined. Only 14 of 88 congeners or congener groups (Nos. 7/9, 34, 69, 96, 88, 136, 175, 167, 197, 193, 169, and 198) were undetectable in every tissue. In contrast, the average concentrations of seven congeners (Nos. 60/56/92/84, 90/101, 99, 77/110, 123/149/118, 132/153/105, and 128) exceeded detection in every tissue; congeners 123/149/118 and 132/153/105 were most concentrated. Overall, there were many congeners that were not measured in the larger sample of 89 fish, but which contributed significantly to the total levels of PCBs in the eight individual eels.

Not all PCB congeners are equally toxic; coplanar structures most similar to that of TCDD are most toxic. Hence, the hazard of consuming eels can be estimated by reference to only a small subset of congeners (TEFs): Nos. 126 (0.1), 169 (0.05), 77 (0.01), 118 (0.001), 105 (0.001), 123 (0.001), 156 (0.001), 157 (0.001), 167 (0.001), and 189 (0.001) (Safe 1990). These congeners were only measured in the eight eels sampled for tissue distribution, and the range of total concentrations within this sample (0.097–1.427 $\mu g \cdot g^{-1}$) was lower than the range of values for the more extensive data set on carcasses. Unfortunately the analytical techniques used did not completely separate all congeners. Hence, No. 126 coeluted with Nos. 129 and 78 and its contribution to TEQs is likely overestimated. The same is true of Nos. 77, 118, 105, 123, 156, and 157. If these mixtures are treated simply as the congener of interest, the TEQ for all eight fish due to all of the toxic congeners was 0.001885 $\mu g \cdot g^{-1}$ or 1885 parts per trillion (ppt), with 86% of the TEQs contributed by No. 126. Despite the unknown bias in treating a mixture of congeners as pure congener 126, it is likely that PCB congeners in eels pose a hazard for beluga whales.

For fish, far less is known about the toxicity of PCB congeners and only a few TEFs are available to predict their hazards. Furthermore, some congeners that are not considered toxic in mammals have significant effects on fish. For example, congener 81 induces the mixed function oxygenase enzymes of fish (Janz and Metcalfe 1991) and is embryotoxic (C. Metcalfe, Trent University, Peterborough, Ont., personal communication). This congener was not included in our analyses but it is not often found in environmental samples (M. Servos, Department of Fisheries and Oceans, Burlington, Ont., personal communication). For other congeners of known toxicity, No. 169 was not detectable and No. 126 (fish TEF = 0.005) coeluted with Nos. 129 and 178, as did No. 77 (fish TEF = 0.002) with No. 110. Hence, there is not a solid basis for judging the hazards of toxicity. Assuming that all of the mixtures containing congeners 126 and 77 could be assigned to these congeners, and that there are no others toxic to fish, the geometric mean TEQs in muscle and gonads were 0.000117 $\mu g \cdot g^{-1}$ (117 ppt) and 0.000130 $\mu g \cdot g^{-1}$ (130 ppt), respectively.

PCDDs and PCDFs were found only at very low concentrations, and many congeners were not detected (Table 3). The most toxic congener, 2,3,7,8-TCDD, was detected only once, at 0.0000014 $\mu g \cdot g^{-1}$ (1.4 ppt), well below the guideline of 0.000020 $\mu g \cdot g^{-1}$ (20 ppt) (Table 2). The only congeners detected frequently were hepta- and octachlorinated PCDDs and PCDFs, at levels up to 0.00012 $\mu g \cdot g^{-1}$ (120 ppt). Hepta- and octachlorinated congeners are the most commonly observed in environmental samples, but of least concern toxicologically, being 100- to 1000-fold less toxic than 2,3,7,8-TCDD (Table 3). Other tetra-, penta-, hexa-, and heptachlorinated congeners were also observed, but even on the basis of total homologues, concentrations were low relative to the guideline in Table 2. Based on the mammalian TEFs for PCDDs and PCDFs (Table 3), TEQs in eels ranged from 0.0000001 to 0.0000011 $\mu g \cdot g^{-1}$ (0.1–1.1 ppt), levels far below the guideline of 20 ppt (Table 2).

The hazard of dioxins was difficult to judge for eels. The tissue dose associated with TCDD toxicity to rainbow trout (*Oncorhynchus mykiss*) embryos is 0.000065 $\mu g \cdot g^{-1}$ (65 ppt), about 50 times higher than levels of TCDD found in eels (Table 3). However, there are no TEFs for the other congeners measured, so that TEQs cannot be calculated. For other tetra-, penta-, and hexachlorinated PCDDs and PCDFs, TEFs for fish range from 0.028 to 0.73 (Walker et al. 1991). If these are applied to the congeners of Table 3, the total TEQs do not approach or exceed 65 ppt.

No eels were found with concentrations of PAHs above the limits of detection of 0.01 $\mu g \cdot g^{-1}$; hence, no results are

TABLE 3. Levels of PCDDs and PCDFs ($pg \cdot g^{-1}$) in homogenates pooled from two eels per week or site. Congeners that did not exceed the detection limit (1.0 $pg \cdot g^{-1}$) are listed in footnote "a". Toxic equivalent factors (TEFs) express the toxicity of each congener as a fraction of the toxicity of 2,3,7,8-TCDD (NATO/CCMS 1988).

Congener	Kamouraska, week: 1	2	3	4	5	6	7	Rivière aux Pins, week 1	TEF
2,3,7,8-Tetrachlorodibenzo[*p*]dioxin	<1	<1	1	<1	1.4	<1	<1	<1	1.0
1,2,3,6,7,8-Hexachlorodibenzo[*p*]dioxin	1.1	<1	<1	3	1	<1	2.3	<1	0.1
1,2,3,4,6,7,8-Heptachlorodibenzo[*p*]dioxin	6	<1	6	6	6	9	6	5	0.1
1,2,3,4,5,6,7,8,9-Octachlorodibenzo[*p*]dioxin	55	91	52	40	120	76	45	45	0.001
Sum of tetrachlorodioxins	<1	<1	1	1.1	1.4	<2	<1	<1	
Sum of hexachlorodioxins	1.1	<1	<1	8.9	2.4	<1.6	3.2	<1	
Sum of heptachlorodioxins	7.3	2.1	9.3	3.1	1.1	1.3	9.3	7.6	
1,2,3,4,6,7,8-Heptachlorodibenzofuran	2	2	3	2	2	4	2.6	2	0.01
1,2,3,4,6,7,8,9-Octachlorodibenzofuran	12	46	14	10	64	18	12	10	0.001
Sum of tetrachlorofurans	17	6	11	3	4	7	6	<19	
Sum of pentachlorofurans	20	6	10	9	11	6	10	<3.9	
Sum of heptachlorofurans	2	2	3	2	3	4	3	2.2	

[a]1,2,3,7,8-Pentachlorodibenzo[*p*]dioxin, 1,2,3,4,7,6-hexachlorodibenzo[*p*]dioxin, 1,2,3,7,8,9-hexachlorodibenzo[*p*]dioxin, 2,3,7,8-tetrachlorodibenzofuran, 1,2,3,7,8-pentachlorodibenzofuran, 2,3,4,7,8-pentachlorodibenzofuran, 1,2,3,4,7,8-hexachlorodibenzofuran, 1,2,3,6,7,8-hexachlorodibenzofuran, 2,3,4,6,7,8-hexachlorodibenzofuran, 1,2,3,7,8,9-hexachlorodibenzofuran, and 1,2,3,4,7,8,9-heptachlorodibenzofuran.

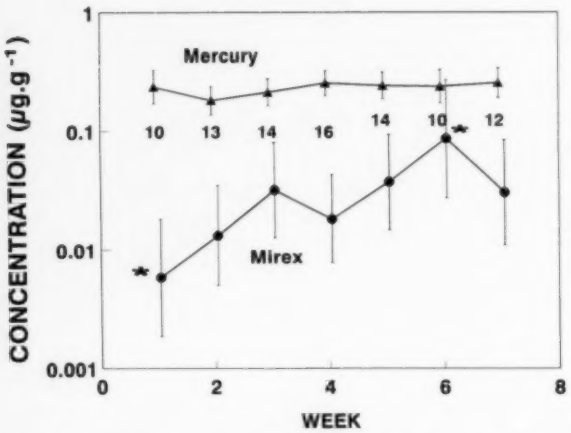

FIG. 2. Geometric mean concentrations and 95% confidence limits of mercury and mirex in carcasses of eels from Kamouraska, September 17 – November 1, 1990. The level of mirex in week 6 was statistically different ($p < 0.05$) from that in week 1. The numbers refer to the sample size for both mercury and mirex analyses.

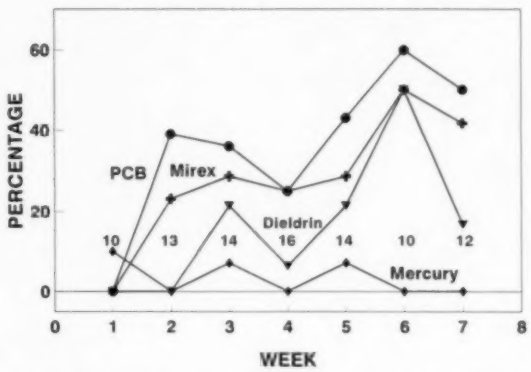

FIG. 3. Percentage of eels sampled at Kamouraska between September 17 and November 1, 1990, that contained PCB, mirex, dieldrin, and mercury at concentrations exceeding the Canadian Guidelines for Human Consumption. The numbers refer to sample size.

given and no conclusions can be drawn regarding variations in levels among sites, over time, among tissues, or among sizes of fish.

A wide range of chlorinated pesticides was detectable in migrating eels, although concentrations varied considerably (Table 1). Lindane, heptachlor, α-BHC, and aldrin were generally close to or below the detection limits of 0.001 $\mu g \cdot g^{-1}$. Heptachlor epoxide, a metabolite of heptachlor, was more abundant, with concentrations in individual fish between 0.003 and 0.06 $\mu g \cdot g^{-1}$, a range that also applied to HCB, oxychlordane, and, for the most part, 1- and 2-chlordane and endrin. Dieldrin was measurable in all samples but one, with concentrations as high as 0.28 $\mu g \cdot g^{-1}$. The most concentrated pesticides were DDT and its metabolites, and the sum of all DDT congeners ranged from 0.028 to 3.312 $\mu g \cdot g^{-1}$. Dieldrin was the only pesticide to exceed the guideline for human consumption; of 89 fish sampled at Kamouraska, 14 (15.7%) exceeded the guideline of 0.1 $\mu g \cdot g^{-1}$ ("other pesticides", Table 2).

Mirex varied from nondetectable to 0.312 $\mu g \cdot g^{-1}$, and the number of fish exceeding the guideline was 26, or 29% of the 89 fish analyzed. Mercury was the only metal measured in a large number of fish. Concentrations ranged from 0.02 to 0.54 $\mu g \cdot g^{-1}$, and only 2.3% of fish exceeded the guideline (Table 2).

Taken together, these data indicate a likely hazard of toxicity due to PCB congeners for both beluga and eels, but little or no hazard due to PCDDs and PCDFs. Assuming that the guidelines to protect human health apply to other mammals, there may also be hazards to beluga from dieldrin, mirex, and mercury.

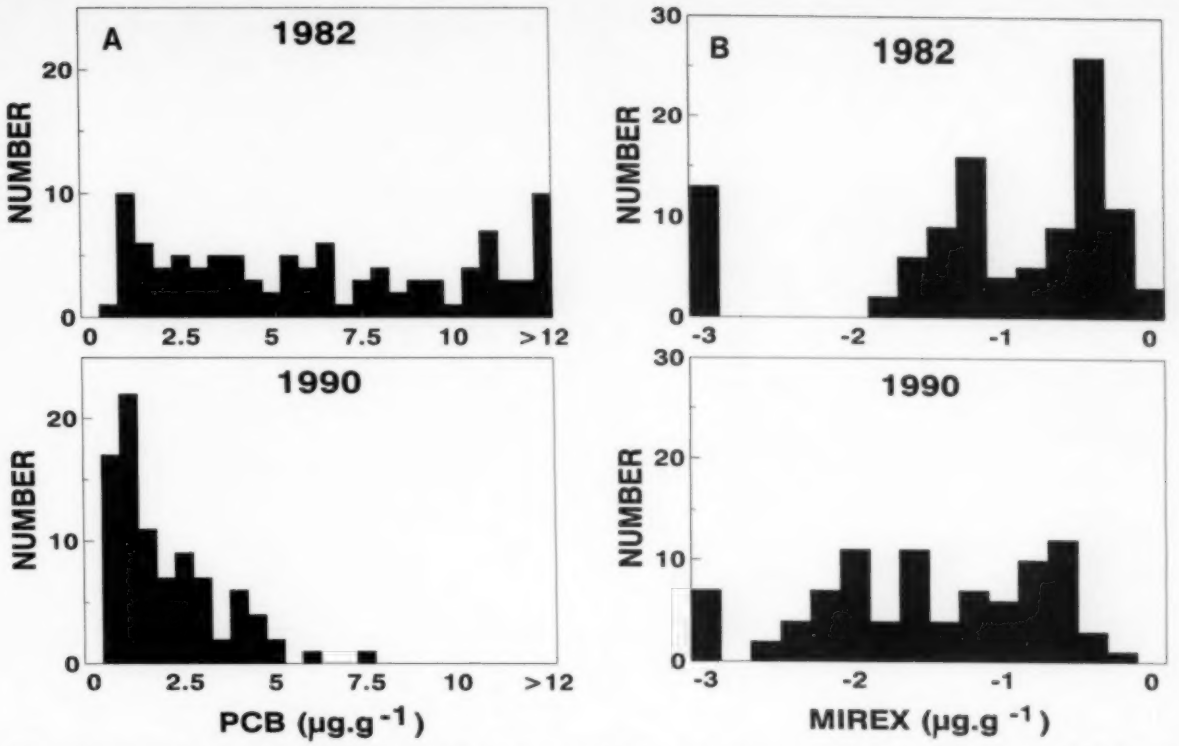

FIG. 4. Frequency distribution of (A) PCB and (B) mirex concentrations in the carcasses of eels sampled at Kamouraska during the annual fishery in 1982 (N = 104) and 1990 (N = 89). For PCBs, the number of fish at 12 $\mu g \cdot g^{-1}$ represents those animals with 12 $\mu g \cdot g^{-1}$ or *greater*; some had concentrations in excess of 20 $\mu g \cdot g^{-1}$. The data for mirex are presented on a log scale to accommodate the large range of values.

Weekly Variations in Chemical Levels

The variations among weeks of concentrations of organic compounds were not consistent with the null hypothesis. Concentrations of mirex, HCB, heptachlor, heptachlor epoxide, aldrin, endrin, and PCB congeners 28 varied significantly among weeks ($p < 0.05$; Table 1) and exhibited an increasing trend over time. For example, mirex levels increased by more than eightfold from week 1 to 6 (Fig. 2). Other pesticides and PCB congeners did not exhibit significant temporal variability ($p > 0.05$; Table 1), although the patterns of concentrations among weeks were similar to those of mirex, with eels from weeks 3 and 6 having the highest levels. The distribution of mercury concentrations was somewhat different, remaining relatively constant over the 7 wk; the small changes were not significantly different and supported the null hypothesis (Fig. 2). If the results are expressed as the percentage of fish exceeding the Canadian Guidelines for Human Consumption, the occurrence of more contaminated fish in later weeks is again apparent, except for mercury (Fig. 3).

For most PCDD and PCDF congeners, there was little variation of levels from week to week with the exception of octachlorodibenzo[*p*]dioxin (OCDD) and octachlorodibenzofuran (OCDF). Concentrations appeared highest in weeks 2 and 5 and least in week 4, although the statistical significance of the differences could not be tested with only one sample per week (Table 3).

Annual Variations in Chemical Levels

Compared with 1982, concentrations of mirex and PCBs in 1990 were significantly lower; the null hypothesis was not supported. Desjardins et al. (1983a, 1983b) reported arithmetic mean levels of PCB and mirex of 6.32 and 0.196 $\mu g \cdot g^{-1}$, respectively, in eels sampled throughout the St. Lawrence in 1982. If these data are recalculated as geometric means to reflect log-normal distributions, the levels of PCBs and mirex from *all* eels in 1982 were 3.47 and 0.099 $\mu g \cdot g^{-1}$, respectively. These data included 397 eels sampled from several estuarine and freshwater sites and the concentrations exceeded the geometric means and 95% confidence limits for PCB and mirex of 1.23 (1.02–1.48) and 0.025 (0.017–0.036) $\mu g \cdot g^{-1}$ found in 1990 at Kamouraska (N = 89).

This may not be a valid comparison, however, since the 1982 samples were taken only once, on October 5, 1982, while the 1990 results encompassed 7 wk of sampling, but only at one site in the estuary. If the comparison is restricted to eels sampled in the estuary at Kamouraska during the first week in October of each year, the appropriate geometric mean levels (and 95% confidence limits) of PCB and mirex for 1982 are 4.54 (3.79–5.44) and 0.073 (0.05–0.11) $\mu g \cdot g^{-1}$, respectively (N = 104). The levels measured in 1990 of 1.43 (0.96–2.14) and 0.032 (0.014–0.072) $\mu g \cdot g^{-1}$ (N = 14) represent declines of 68 and 56% for PCB and mirex, respectively.

TABLE 4. Ranges of concentrations ($\mu g \cdot g^{-1}$) of eight PCB congeners, pesticides, mirex, and mercury in eels from a reference site (Rivière aux Pins), the north shore (Saint-Irénée), and the south shore (Cacouna). The *F*-statistics indicate whether there was a significant difference of chemical levels between each of the three sites and Kamouraska (Table 1) for the corresponding weeks of sampling. An asterisk indicates statistical significance at the 0.05 probability level.

	Reference		North shore		South shore	
Compound	Range	*F*	Range	*F*	Range	*F*
N	7		13		15	
PCB congeners						
28	<0.001	5.7*	<0.001–0.055	3.6	<0.001–0.027	0.9
52	<0.001	179.7*	0.025–0.141	0.1	0.012–0.159	2.2
101	<0.001–0.001	203.6*	0.018–0.131	2.8	0.006–0.202	1.1
118	0.001–0.004	175.3*	0.048–0.284	2.2	0.010–0.331	0.5
153	0.001–0.005	165.3*	0.060–0.520	1.4	0.021–0.528	0.1
137	<0.001	17.4*	<0.001–0.031	1.4	<0.001–0.031	0.4
138	<0.001–0.004	138.5*	0.054–0.473	1.6	0.017–0.473	0.1
180	<0.001–0.002	176.9*	0.025–0.391	1.1	0.001–0.358	0.1
Sum of congeners	0.005–0.011	288.8*	0.230–1.374	0.6	0.070–2.042	0.3
Total Arochlor 1254	0.002–0.022	138.6*	0.537–5.263	1.4	0.142–5.391	0.3
Pesticides						
p,p'-DDE	<0.001–0.020	14.6*	0.055–1.121	0.9	0.043–1.194	0.1
p,p'-DDD	<0.001–0.008	92.5*	0.015–0.121	2.2	0.010–0.113	6.4*
p,p'-DDT	<0.001–0.007	43.2*	0.005–0.182	0.3	0.005–0.176	1.8
Total DDT	0.007–0.026	30.3*	0.078–1.325	0.5	0.060–1.483	0.5
Hexachlorobenzene	0.001–0.002	22.8*	0.006–0.079	1.9	0.002–0.063	0.0
α-BHC	0.002–0.004	22.8*	0.004–0.007	1.3	0.003–0.006	9.3*
Heptachlor	<0.001	—	<0.001–0.001	2.4	<0.001	—
Heptachlor epoxide	0.001–0.002	67.0*	0.005–0.028	0.2	0.002–0.029	5.9*
Lindane	<0.001–0.001	137.3*	0.001–0.002	1.0	0.001–0.002	3.7
Aldrin	<0.001	2.2	<0.001–0.001	0.0	<0.001–0.001	2.9
Endrin	<0.001–0.001	5.8*	<0.001–0.012	1.3	<0.001–0.018	0.5
Dieldrin	0.001–0.004	65.3*	0.016–0.125	0.0	0.004–0.128	6.3*
Oxychlordane	<0.001–0.001	90.9*	0.002–0.024	0.1	0.002–0.025	1.5
1-Chlordane	<0.001–0.001	60.6*	0.002–0.025	0.9	0.001–0.019	1.8
2-Chlordane	<0.001–0.001	127.4*	0.006–0.087	0.1	0.004–0.079	1.8
Total pesticides	0.024–0.044	67.1*	0.152–1.479	0.3	0.087–1.778	0.9
Mirex	<0.001–0.001	14.4*	0.008–0.474	2.8	0.001–0.337	1.5
Mercury	0.134–0.321	0.1	0.074–0.946	0.5	0.050–0.990	1.5

Since eels move and appear to mix together, these comparisons contain a bias due to the unknown percentage in each sample of eels from different freshwater ecosystems. Since the estuary fishery occurs during the same few weeks every year, the comparison between 1982 and 1990 for eels caught only at Kamouraska in the same calendar week is likely the most consistent way to assess temporal trends.

Pesticide levels also appear to have declined since 1982, with the possible exception of dieldrin. Castonguay et al. (1989) reported that the arithmetic mean levels of *p,p'*-DDE in eels sampled in 1982 from six locations ranged from 247 to 753 $\mu g \cdot g^{-1}$, while dieldrin ranged from 18 to 129 $\mu g \cdot g^{-1}$. At Kamouraska, the levels of *p,p'*-DDE and dieldrin were 753 and 18 $\mu g \cdot g^{-1}$, respectively. In 1990 at Kamouraska, the ranges of geometric means for *p,p'*-DDE and dieldrin were 45–85 and 25–65 $\mu g \cdot g^{-1}$, respectively.

An alternative comparison of contamination between years is the percentage of fish that exceed the current guidelines for protecting human health. For PCBs, the percentage of eels at Kamouraska that exceeded 2.0 $\mu g \cdot g^{-1}$ in 1982 was 80%, more than double the 36% that exceeded the limit in 1990. Similarly, for mirex, the percentage of eels exceeding 0.1 $\mu g \cdot g^{-1}$ was 52% in 1982 and 29% in 1990. The frequency distributions of PCB and mirex in 1982 and 1990 demonstrate the absence in 1990 of the highly contaminated fish typical of 1982 (Fig. 4). For pesticides, the percentage of fish exceeding the guidelines for eels sampled at Kamouraska actually increased slightly from 1982 to 1990, although there was a twofold decrease on an "all-eel" basis (Table 2). All fish that exceeded the guidelines only exceeded the limit for dieldrin; no other pesticide was above the limit. The percentage of fish with excessive levels of mercury declined by about four times, although the absolute level and the relative decline were small.

Spatial Variations in Chemical Levels

The levels of PCBs, mirex, and most pesticides in eels collected at Saint-Irénée, on the north shore of the estuary, and at Cacouna, on the south shore, were little different from levels in eels from Kamouraska caught in the corresponding weeks (Table 4; Fig. 5). For example, the geometric mean level of Aroclor 1254 at Cacouna on October 18, 1990, was 1.143 $\mu g \cdot g^{-1}$, while the level on October 16 at

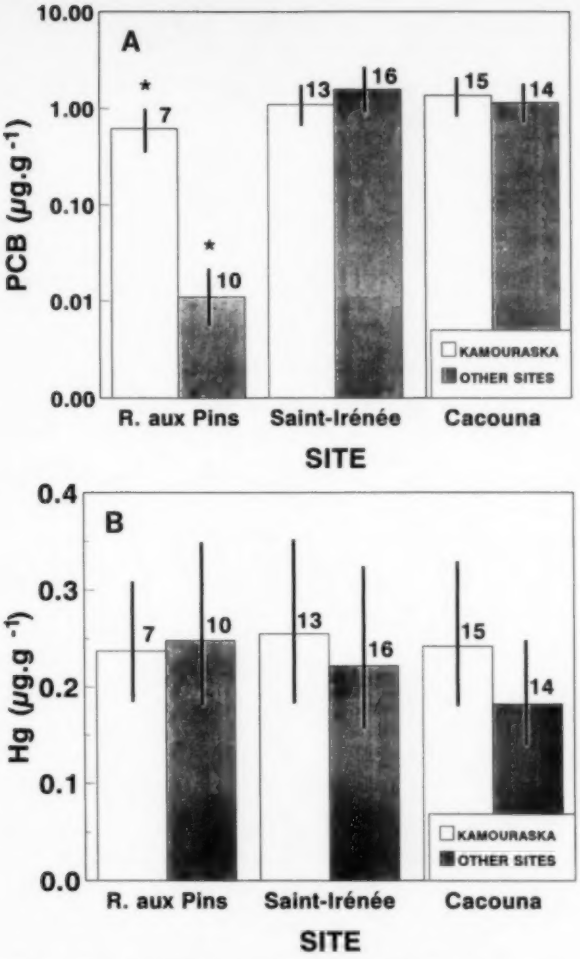

FIG. 5. Paired comparisons of (A) PCBs and (B) mercury concentrations in eels. Paired comparisons used Kamouraska as a reference point to account for variations in concentrations among weeks (Fig. 2). There were no significant differences ($p > 0.05$) except for the comparison of PCB levels between Kamouraska and Rivière aux Pins. Numbers refer to sample sizes and error bars represent 95% confidence limits.

Kamouraska was similar at 1.359 $\mu g \cdot g^{-1}$. However, compared with Kamouraska, there were small but significant differences in the concentrations of *p,p'*-DDD, α-BHC, heptachlor epoxide, and dieldrin, with levels at Cacouna lower than at Kamouraska.

In contrast, contamination of eels from Rivière aux Pins by organic compounds was much lower, with the majority of PCB congeners and pesticides at concentrations at or below the detection limit. The compounds with the highest concentrations at Rivière aux Pins were Aroclor 1254 and *p,p'*-DDE. Nevertheless, these levels were more than 50 times lower than at Kamouraska. PCDDs and PCDFs in eels from Rivière aux Pins were also low, equivalent to the lowest levels observed at Kamouraska and generally below the limit of detection (Table 3). The only exception to low levels of contamination at Rivière aux Pins was mercury. Concentrations ranged from 0.13 to 0.32 $\mu g \cdot g^{-1}$, and the average (0.248 $\mu g \cdot g^{-1}$) was equivalent to that at Kamouraska during the same week (0.240 $\mu g \cdot g^{-1}$).

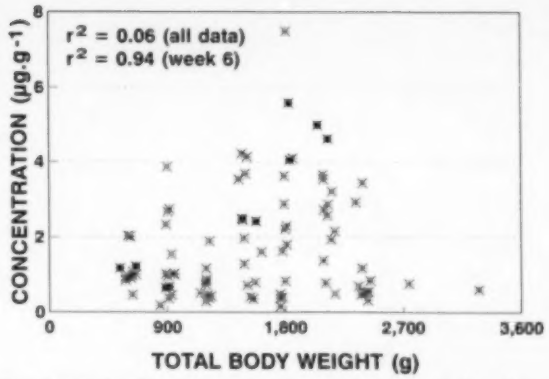

FIG. 6. Relationship between total pesticide concentrations (asterisks) in carcasses and whole-body wet weight for 89 eels sampled at Kamouraska in 1990. Fish sampled in week 6 are shown as solid squares.

Size Effects

Fish weight was not consistently correlated with chemical levels. For all fish sampled at Kamouraska, there were no systematic trends in levels of PCBs (total or individual congeners), mirex, mercury, and pesticides such as *p,p'*-DDE, HCB, α-BHC, aldrin, heptachlor epoxide, dieldrin, and endrin. However, there were weak, but significant positive relationships for *p,p'*-DDD, *p,p'*-DDT, total DDT, heptachlor, oxychlordane, chlordane, and total pesticides (e.g., Fig. 6, total pesticides; $r^2 = 0.06$, $p = 0.0181$, $N = 89$). In contrast, relationships for specific weeks were much stronger (e.g., Fig. 6, total pesticides, week 6; $r^2 = 0.94$, $p = 0.0001$, $N = 10$). These stronger relationships were evident for all organochlorine contaminants and were most frequently found in weeks 4 and 6.

Tissue Distribution

There were few differences in the patterns of distribution of PCB congeners among tissues; those that were low in one tissue were low in all tissues, and vice versa. Total PCBs did not vary significantly among tissues ($p = 0.55$), probably due to the small sample size, but the calculated levels in whole body were most similar to those in carcass (Fig. 7). All pesticide concentrations varied among tissues in much the same way and are exemplified by "total DDT" (Fig. 7). Concentrations were highest in the gonads and least in the head, and the differences among tissues were parallelled by differences in tissue lipid levels. When the concentrations in whole body were calculated, they differed little from those in carcass ($p > 0.05$).

Due to the high concentration of bone in the head, difficulties were encountered in preparing extracts suitable for metal analyses. Consequently, there are data only for carcass, viscera, and gonad; whole-body concentrations could not be calculated. For cadmium and copper, concentrations were highest in viscera, about 15 times higher than in carcass or gonad (Fig. 8). Chromium was also more concentrated in viscera, but the levels averaged only about 70%

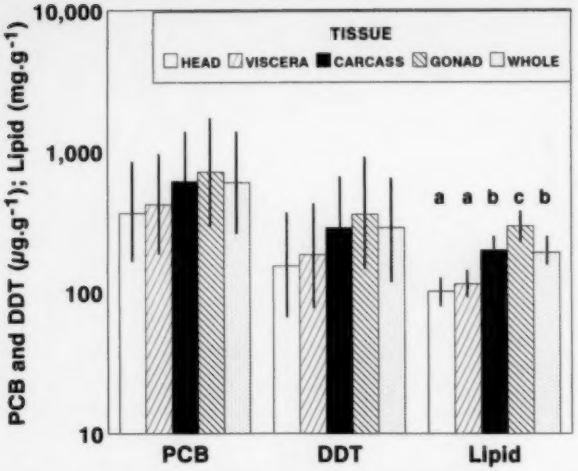

FIG. 7. Distribution of PCBs, total DDT, and lipid in five tissues of eight eels sampled at Kamouraska in 1990. Vertical bars are 95% confidence limits and letters signify differences among treatments; treatments with the same letter are not statistically different ($p > 0.05$). There were no differences among means for PCBs or for DDT.

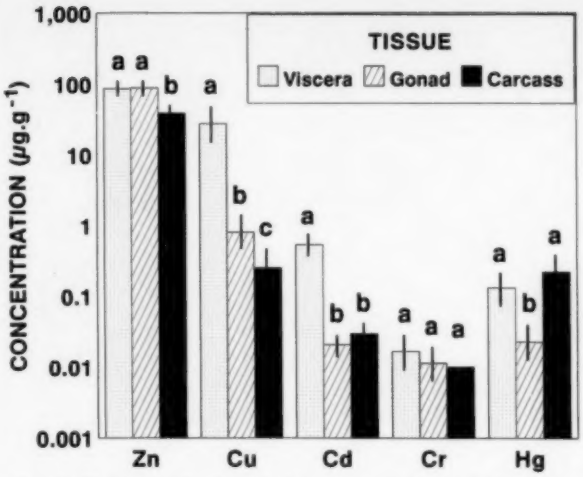

FIG. 8. Tissue distribution of zinc, copper, cadmium, chromium, and mercury in three tissues of eight eels sampled at Kamouraska in 1990. Vertical bars are 95% confidence limits and letters signify differences among treatments; treatments with the same letter are not statistically different ($p > 0.05$).

higher than in carcass and gonad. Zinc concentrations in viscera and gonad were the same, and more than double those in carcass. Mercury differed from all other metals, in that concentrations were highest in carcass, almost twice those found in viscera, and 10 times those found in gonad. With the exception of chromium, the differences among the three tissues in levels of metals were highly significant ($p<0.0001$ in all cases); there were no differences for chromium ($p=0.28$), probably because many concentrations were less than the limits of detection.

Discussion

These analyses have demonstrated the occurrence of a wide variety of xenobiotic compounds in silver eels migrating through the estuary of the St. Lawrence River. These compounds included not only the long-recognized chemicals such as DDT and mercury, but also some highly toxic congeners of PCBs, PCDDs, and PCDFs. However, PCDDs and PCDFs were not present in high concentrations, suggesting little or no threat to the health of either beluga whales or eels. By comparison, the TCDD equivalents in four individual eels collected from Lake Ontario and the Upper St. Lawrence River in May 1990 ranged from 0.000001 to 0.0000067 $\mu g \cdot g^{-1}$ (1–6.7 ppt) (D.M. Whittle, Department of Fisheries and Oceans, Burlington, Ont., personal communication).

In contrast, measured levels of specific PCB congeners in eel tissues may be hazardous both to mammalian consumers and to the eels themselves. The sum of TEQs (1185 ppt for mammals; 117 ppt for fish) exceeded the Canadian Guidelines for Human Consumption of 20 ppt and the threshold for chronic toxicity to fish of 65 ppt, based on sensitivity of eggs and embryos of trout (Walker et al. 1991). Other studies also implicate pesticides and PCBs as toxic to juvenile stages of salmonids, through correlations of survival with parental contamination for Great Lakes fish and for juvenile fish exposed experimentally, either directly or through the parents. Concentrations in eggs associated with increased rates of mortality up to the feeding stage ranged from about 1 to 10 $\mu g \cdot g^{-1}$ for both PCBs and DDT, whether the exposure was natural or experimental (Giesy et al. 1986). Detailed chemical and statistical analysis of chemical concentrations and juvenile mortality in chinook salmon (*Oncorhynchus tshawytscha*) from the Great Lakes showed that mortality was associated with PCBs and toxaphene, and not with other pesticides. The levels of total PCBs in eggs were about 9.16 $\mu g \cdot g^{-1}$, well within the range of concentrations associated with mortality in other studies, while DDT was about 1.2 $\mu g \cdot g^{-1}$, a level at the lower end of the toxic range (Giesy et al. 1986).

PCB and DDT concentrations in eel gonads were about 17 and 28% higher than in carcasses (Fig. 7). Since measured PCB and DDT concentrations in carcasses ranged up to 7.5 and 2.2 $\mu g \cdot g^{-1}$, respectively, chemicals in eggs of some individuals could exceed thresholds of toxicity, assuming that eel larvae are as sensitive to chemicals as salmonid larvae. It should also be recognized that eel gonads were only partially developed at the time of capture. During migration, females will not feed and will mobilize lipid reserves, both for migration and for further egg development. Therefore, chemical levels in eggs could be higher at hatch than measured during this survey, increasing the likelihood of chemical toxicity to larvae. The once-in-a-lifetime spawning of eels makes this species particularly susceptible to recruitment failure and aggravates chemical accumulation, since chemicals are not lost with gametes during repeat spawning, as would occur with some salmonids (Larsson et al. 1991).

There were significant increases in chemical concentrations within the 7-wk fishery for eels caught at Kamouraska. The nonhomogenous distribution was primarily related to time, since eels caught at other locations in the estuary had similar levels of contamination as eels caught at Kamouraska in the corresponding week. The trend of increasing levels of

contamination with time corresponds to observations of increasing frequencies of pathologies towards the end of the eel migration (C.M. Couillard and P.V. Hodson, unpublished observations; Homer 1986). This could indicate either that contaminants and pathologies slow the migration of eels or that eels migrating from areas that are particularly contaminated, such as Lake Ontario, have a longer distance to cover or are slightly delayed in starting. This latter suggestion implies that eels from different locations are not completely mixed together and that they may migrate in cohesive groups, at least when migration starts.

The temporal variation of contaminant levels indicates that the risk of toxicity to beluga would increase with time during the migration, although there was considerable variability within each sample of eels. For example, in week 7, there was a 13-fold spread in levels of Aroclor 1254, so that both "clean" and "contaminated" eels appear to migrate together. Therefore, risk could be expressed as a function of both the level of contamination and the percentage of eels contaminated.

While eels from the St. Lawrence contain important levels of organic contaminants, levels are declining, with less contamination now than in the early 1980s (except for dieldrin). The decline in chemical levels of eels is in sharp contrast with the relatively constant levels of contamination observed in Great Lakes forage and predatory fish since 1982. In Lake Ontario, an important area for the feeding and growth of juvenile eels, levels of PCBs, mirex, and pesticides in lake trout (*Salvelinus namaycush*) declined dramatically during the 1970's, but have shown little change since 1980 (Borgmann and Whittle 1991). There are at least two possible explanations. Eels spend 8–12 yr in Lake Ontario (Castonguay et al. 1994) and their contamination at the time of capture represents their lifetime exposure. Hence, an eel migrating in 1982 represents 1970–82, while an eel migrating in 1990 represents 1978–90. The apparent decline in concentration may therefore reflect the decline in Lake Ontario contamination between the 1970's and 1980's. The relatively stable levels typical of the 1980's may show up in the future as stability in levels of chemicals in the mid- to late 1990's. An alternative explanation is that eels sampled at Kamouraska represent a mixture of eels from many sources, so that declines in contamination reflect environmental improvements in regions outside Lake Ontario. However, since the majority of migrating adult eels originate from Lake Ontario (Dutil et al. 1985), this is less likely.

The continuing high levels of dieldrin could be due to use patterns. While most persistent organochlorine pesticides such as DDT were banned for use in Canada in the 1970's, dieldrin is still used to control insect pests of livestock (C. Charbonneau, Health and Welfare Canada, Ottawa, Ont., personal communication). Since both the Lake Ontario and southern Quebec watersheds support intensive agriculture, there may be a continued release of dieldrin.

Compared with eels from a reference stream, those migrating from the St. Lawrence River drainage basin were much more contaminated with chlorinated organic compounds; in contrast, mercury showed little difference. The contamination of eels with organic chemicals likely varies according to specific sources of pollution in areas where they grow to maturity. Mercury, however, appears more widespread and equally distributed, indicating either a natural source or dispersion by atmospheric transport. Although the levels approach or occasionally exceed the guideline of 0.5 $\mu g \cdot g^{-1}$, this does not mean that all ecosystems are heavily contaminated. Rather, it indicates the importance of bioaccumulation by a long-lived, predatory species.

Spatial variability of chemical levels in eels within the estuary was much less than temporal variability. In fact, the paired comparison of each site with Kamouraska was necessary due to obvious changes in chemicals levels over 7 wk of sampling (Fig. 2). The small but significant differences in levels of some pesticides between Cacouna and Kamouraska imply differences in exposure of the two groups of eels, despite capture within the same week and within 50 km. These small differences may or may not be real, since we made 56 comparisons of chemical levels between two pairs of sites. At a probability level of 0.05 for Type I error, these differences could simply be coincidental. If we assume that they are real, fish caught at Kamouraska and Cacouna may represent different feeding areas or ecosystems, varying primarily in contamination by agricultural chemicals. This, as well as the weekly changes at Kamouraska, argues that eels of different origin may be migrating as relatively cohesive groups.

Variations in parasite levels in fish support the notion of cohesive groups. C.M. Couillard (unpublished data) found that the species and numbers of intestinal parasites in eels from Cacouna on October 18, 1990, matched those observed in eels from Kamouraska on October 9, the week previous, and not those in eels from Kamouraska on October 16. Since Cacouna is about 50 km further downstream, the site-to-site comparison should probably be based on eels captured in these two different weeks, rather than in the same week. From Table 4, eels from Cacouna differed from eels from Kamouraska in their levels of *p,p'*-DDD, α-BHC, heptachlor epoxide, and dieldrin. However, comparing the geometric mean levels among Cacouna (October 18), Kamouraska (October 16), and Kamouraska (October 9), the same differences are seen, regardless of week (e.g., *p,p'*-DDD: 0.031, 0.061, and 0.060 $\mu g \cdot g^{-1}$).

The utility of mirex to discriminate stocks, as described by Dutil et al. (1985) and Castonguay et al. (1989) for eels sampled in 1982, may have been lost; we were unable to separate groups of eels on the basis of mirex concentrations. Plots of mirex versus PCB demonstrate similar relationships between the two for eels caught in 1982 (Fig. 9A) and 1990 (Fig. 9B). If mirex were present only in Lake Ontario and the St. Lawrence River, as appeared to be the case in 1982 (Castonguay et al. 1989), there should be at least three groups of eels evident. The first would be those with high levels of both PCBs and mirex, typical of Lake Ontario and St. Lawrence River ecosystems. The second would be eels having low levels of both, typical of uncontaminated tributaries. The third would be eels with high levels of PCB, but low levels of mirex, typical of fish from developed tributaries such as the Ottawa River, with contamination by PCBs but not mirex. For 1982 and 1990, both the first and second groups are evident. For 1982, the third group is represented by "outliers", fish with high levels of PCB and low levels of mirex, falling to the lower right of the main groups of eels (Fig. 9A). This third group is much less evident in 1990, probably due to the lower overall levels of contamination (Fig. 9B). The continuous distribution of mirex concentrations in both years implies that mirex is also more widespread than previously thought, probably

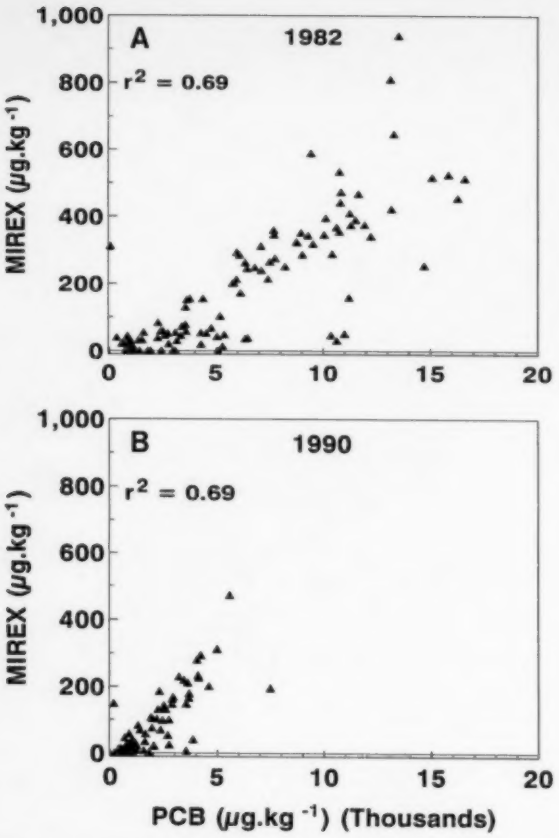

FIG. 9. Relationship between levels of mirex and PCBs in eels sampled at Kamouraska in (A) 1982 (N = 104) and (B) 1990 (N = 89).

through atmospheric transport, since mirex is now found at low levels in Arctic ecosystems (Muir et al. 1990, 1992).

While eels of the St. Lawrence contain high levels of chemicals, they are no more contaminated than those from other North American watersheds receiving industrial or urban waste. Eels in estuaries and coastal waters of New Jersey in the late 1970's and early 1980's averaged from 1.52 to 7.2 µg PCB.g^{-1} as Arochlor 1254, and values varied regionally according to sources (Kennish et al. 1992). There was little change with time, although monitoring spanned only a few years. Eels in the St. Lawrence watershed may be less contaminated than some European eels (*Anguilla anguilla*), such as those from the River Mersey and Liverpool Bay in England, which averaged 7.38 µg·g^{-1} in muscle; some values were as high as 34.9 µg·g^{-1} (Johnston et al. 1991). For European eels from the River Rhine, levels of congener 138 were 1.41 ± 0.56 µg·g lipid^{-1} (Duursma et al. 1991) compared with values in this study that ranged from 0.003 to 0.42 µg·g^{-1} (N = 8) as calculated from levels of lipid and congener 138.

An apparent anomaly in these data was the poor relationship between contaminant levels and fish size, in contrast with other fish species from Lake Ontario (Baumann and Whittle 1988). Larsson et al. (1991) reported an age–concentration relationship for European eels of a wide range in ages captured by trawling in a freshwater lake. The age effect, which would translate into a size effect, could be accounted for by changes in lipid concentrations with growth. However, within one age group, there was considerable variability in chemical levels, a situation similar to that of migrating mature eels. The size–concentration relationship in the current data was much stronger when considered on a "within-week" basis, suggesting again that eels migrate in cohesive groups. If true, the weak relationship on an "all-fish" basis may be explained by different levels of contamination among groups of diverse origin. When mixed, the size–concentration relationship would be impossible to discriminate, since eels of the same size would be either highly contaminated or free from contamination. The consistent size–concentration relationships in weeks 4 and 6 may represent periods when eels from one source predominate.

The question of discriminating groups of eels according to their freshwater habitat may ultimately be resolved by additional chemical analyses. While our data strongly suggest distinct groups that mix during migration, attempts to discriminate groups by principal components analyses of the spectrum of contaminants (data not shown) were unsuccessful. One possible reason may simply be the overall decline in concentrations towards the limits of detection. The frequency distribution of PCB, for example, has changed from a relatively flat distribution in 1982, implying a mixture of groups with widely varying concentrations, to one which appears log-normal in 1990 (Fig. 4A), representing a narrower range of concentrations. However, the true distribution and variability are now obscured by the detection limit. The most fruitful approach to discriminating groups will likely be one of sampling closer to the origins of the principal groups during the entire period of the annual migration.

The tissue distribution of mercury and persistent organic chemicals indicates that analysis of carcass (gutted carcass, skin on) is a suitable basis for estimating the hazard to whales of eating whole eels. This is not surprising, given that carcass makes up 80% of the total body mass. Despite higher levels of fat and lipid-soluble contaminants, the viscera did not contribute a great deal to whole-body contamination.

Tissue distribution of mercury differed considerably from that of other metals, showing preferential accumulation in carcass due to the affinity of methyl mercury for protein (Hodson 1988). Other metals were generally most concentrated in the viscera, reflecting either uptake or excretion via the gut, or accumulation where their nutrient functions are expressed. Cadmium is not a micronutrient, but follows the same pattern of distribution as copper, perhaps because it has similar properties and is bound and excreted by the same carrier proteins (e.g., metallothionein) (Hodson 1988).

This paper demonstrates that eels migrating through the St. Lawrence estuary are still contaminated by persistent chlorinated organic compounds, but that concentrations have declined considerably since 1982. While the toxic congeners of dioxins and furans are virtually absent, there may be a hazard to both eels and beluga whales of specific PCB congeners that have dioxin-like toxicity. The risks of this contamination are difficult to judge, since specific congeners coelute with other less potent congeners in the chemical analysis, and specific data on chemical toxicity to eels and beluga are not available. Fortunately, the risk is declining with time as the overall levels of contamination decrease. The

hazard of chemical contamination is well represented by analyses of carcass, is uniform throughout the estuary, and is generally unrelated to fish size. Mass mortalities are no longer seen in eels migrating in the freshwater portion of the St. Lawrence, but some mortalities still occur and the numbers of young eels ascending the eel ladder at Cornwall, Ontario, is declining at the same time as chemical levels are declining. It is possible that levels of chemicals in gonads of female eels are sufficiently high to cause elevated mortality rates in larvae, but there is no experimental support for this hypothesis. Further research and data analyses are required to link the health of eels and beluga, and the status of their populations, to the levels of chemicals in the flesh of eels.

Acknowledgments

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REVIEWS / SYNTHÈSES

Why Is Recruitment of the American Eel, *Anguilla rostrata*, Declining in the St. Lawrence River and Gulf?

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We critically review four potential causes of a drastic decline in juvenile American eels, *Anguilla rostrata*, recruiting to Lake Ontario (81-fold decline from 1985 to 1992) and in juvenile eel densities in tributaries to the Gulf of St. Lawrence. Silver eels from the St. Lawrence River were much more contaminated and had a high prevalence of deformities and lesions than a reference stock although they were on average less contaminated in 1990 than in 1982 (1.12 versus 4.54 $\mu g \cdot g^{-1}$ for PCB; 0.025 versus 0.07 $\mu g \cdot g^{-1}$ for mirex). Lethal toxicity from chemical contamination has been known to occur in St. Lawrence River eels for the past 25–30 yr. Major habitat modifications in the St. Lawrence took place in the 1950's (St. Lawrence Seaway and hydroelectric dams), about 30 yr before recruitment started declining; this long delay argues against these perturbations being primary causes of the decline. There is little evidence that commercial fishing and oceanographic changes caused the decline. Overall, we conclude that we do not know what caused the pronounced recruitment decline. We propose research avenues and hypotheses that may advance understanding and emphasize that because of panmixia, the recruitment decline could be species wide.

Nous examinons quatre causes possibles d'une diminution prononcée du recrutement d'anguilles d'Amérique juvéniles, *Anguilla rostrata*, au Lac Ontario (81 fois de 1985 à 1992) et des densités d'anguilles juvéniles de tributaires du golfe Saint-Laurent. Les anguilles argentées du Saint-Laurent étaient beaucoup plus contaminées et avaient une plus forte prévalence de difformités et de lésions qu'un stock de référence bien qu'elles étaient en moyenne moins contaminées en 1990 qu'en 1982 (BPC : 1,12 versus 4,54 $\mu g \cdot g^{-1}$; mirex : 0,025 versus 0.07 $\mu g \cdot g^{-1}$). Des cas de toxicité létale due à la contamination chimique sont rapportés chez l'anguille du Saint-Laurent depuis 25–30 ans. Les modifications majeures à l'habitat du Saint-Laurent se sont produites durant les années 1950 (voie maritime et barrages hydroélectriques), environ 30 ans avant la baisse de recrutement; ce long délai suggère que ces perturbations ne sont pas responsables de la baisse. Il existe peu d'indications que la pêche commerciale et les changements océaniques aient causé le déclin. Nous concluons que la cause de la baisse est inconnue et proposons des voies de recherche et des hypothèses pour augmenter notre compréhension. Nous soulignons qu'à cause de la panmixie, cette diminution pourrait affecter l'espèce entière.

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A large stock of American eels, *Anguila rostrata*, grows to onset of sexual maturity in the St. Lawrence River drainage basin (Fig. 1) and sustains a fishery of 500–700 metric tons (t). There appears to have been a drastic decline between 1985 and 1992 in the number of juvenile eels (so-called yellow eels) migrating upstream to Lake Ontario at an eel ladder installed at the R.H. Saunders hydroelectric dam located on the upper St. Lawrence River in

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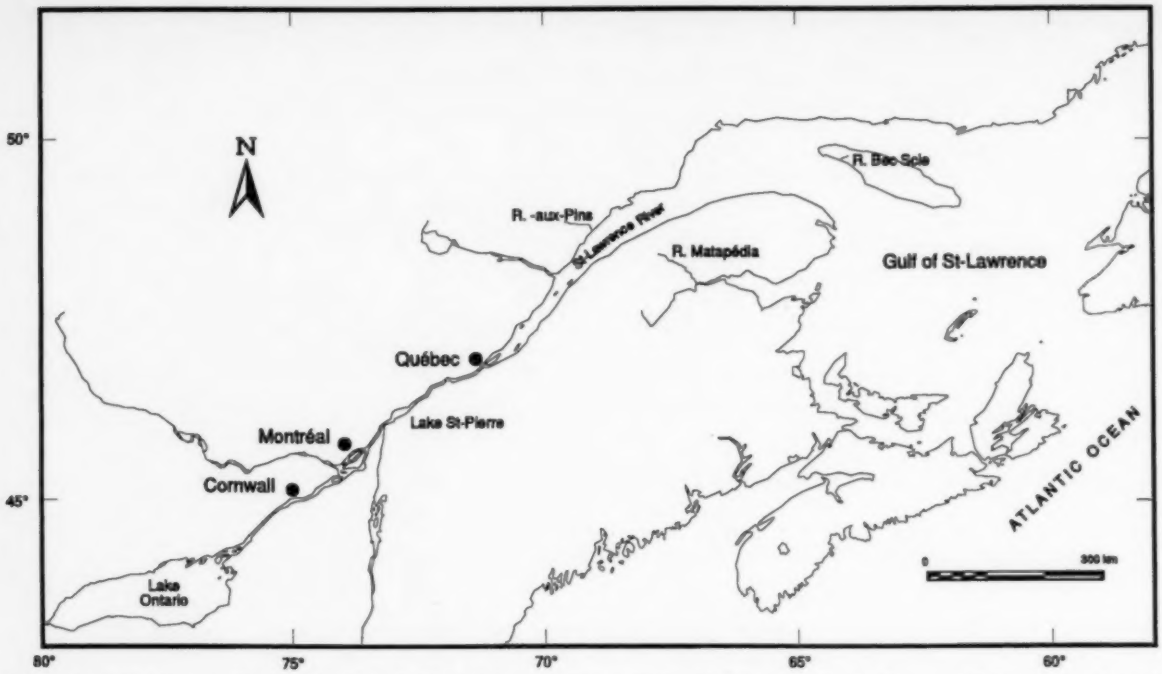

FIG. 1. Map of study area. The Saunders Dam ladder is located in Cornwall (Ontario, Canada). The St. Lawrence fishery is based mostly in the estuary (within 200 km downstream of Québec City) and in Lake St. Pierre, and enlargement of the St. Lawrence River.

Cornwall, Ontario, Canada (Fig. 1). Most juveniles entering the St. Lawrence River eventually settle in Lake Ontario (Dutil et al. 1985), the only Great Lake where eels are commonly found (Scott and Crossman 1973). Concern for this recruitment decline to the St. Lawrence River prompted an investigation of its possible causes.

The implications of such a decline in *Anguilla*, if true, are that recruitment trends in a given area may reflect recruitment trends in other parts of a species' range. Oceanic migrations of eel larvae towards the continents are achieved by drift (Schmidt 1925; Power and McCleave 1983; Helfman et al. 1987; McCleave 1987); hence, larval migrations are greatly influenced by oceanic currents. The magnitude of eel recruitment to a given freshwater area would then depend on both oceanic currents, which have a strong random component, and the number of larvae produced in the Sargasso Sea. Nonrandom fluctuations of recruitment, such as the decline presented here, may reflect trends primarily in the production of *Anguilla* larvae in the Sargasso Sea. Hence, the St. Lawrence River recruitment decline may be representative of recruitment trends of the population (the species) as a whole, especially in the case of the Saunders Dam ladder index, which is based on large sample sizes.

Empirical evidence for synchrony of recuitment trends among different parts of the range of an *Anguilla* species comes from Moriarty (1990). He calculated significant correlations among recruitment indices for the European eel (*Anguilla anguilla*) from 11 European localities. Moreover, a widespread, though not universal, recruitment decline has also been observed during the last decade for the European eel (Moriarty 1990). Recruitment comparisons among different rivers are not possible with the American eel, as the Saunders Dam ladder provides the only long-term recruitment data for this species.

Recruitment variability of the American eel must be examined at the level of the whole species, as genetic evidence shows this species to be a single panmictic (i.e., randomly breeding) population (Williams et al. 1973; Koehn and Williams 1978; Williams and Koehn 1984; Avise et al. 1986, 1990). Consequently, the number of recruits invading a given river in a given year would be related not to the number of adults that left that river 1.5 or more yr before, but to the total number from all geographic areas combined that reached the spawning area in the Sargasso Sea. Only when the number of adult eels from a given river contributes substantially to the total adult reproductive population could there possibly be a cause–effect relationship between the number of adults leaving a river and the number of recruits returning to the same river. Such may be the case for the St. Lawrence River (SLR) eel stock.

The objective of this study is to critically review the evidence of a recruitment decline of the SLR eel stock and to assess published and unpublished information pertaining to possible causes of the decline. Four potential causes were considered: (1) toxicity from anthropogenic chemical contamination, (2) anthropogenic habitat modifications, (3) commercial fishing, and (4) oceanic changes. This study formed part of a program on the impact of contaminants on the health of eels (Hodson et al. 1992, 1994) and the role of eels as vectors of contaminants to beluga whales (*Delphinapterus leucas*) (Hickie et al. 1991). In this study, "stock" refers to a production or a management unit, as defined by Larkin (1972), and as used previously for this species (Dutil et al. 1985; Castonguay et al. 1989). The SLR eel stock is

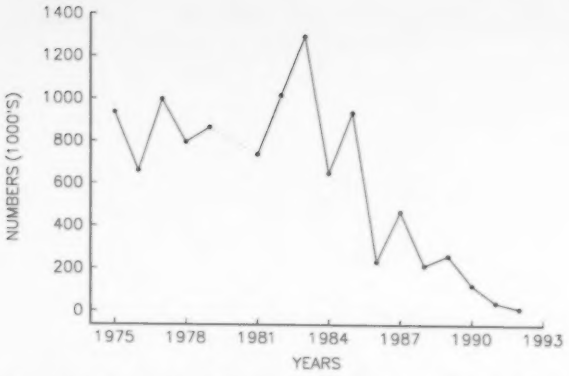

FIG. 2. Numbers of juvenile American eels counted per year at the Saunders Dam eel ladder, as reported by Eckersley (1982), Hendrick (1991), and M.J. Eckersley (unpublished data). Eels were not counted in 1980.

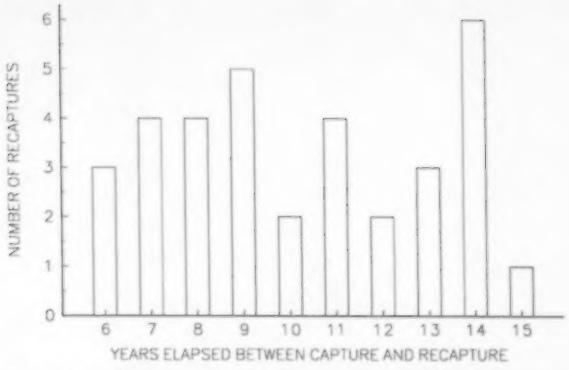

FIG. 3. Number of years spent by eels in Lake Ontario, as indicated by the number of years elapsed between tagging as yellow eels at the Saunders Dam ladder and recaptures as silver eels in the St. Lawrence River fishery (N = 34).

defined here as the group of eels inhabiting Lake Ontario, the St. Lawrence River, and their tributaries.

Life History of the SLR Eel Stock

Reconstructing the SLR eel life history is important for correct interpretation of recruitment variability of this stock. Liew (1974) observed false annuli on otoliths of captive American eels and Michaud et al. (1988) showed that ring formation on otoliths of wild juvenile American eels is probably not annual. No age determinations were attempted in this study; other sources of information, mostly results of unpublished tagging studies, were used to infer the life cycle of the SLR eel.

An American eel leptocephalus ("leptocephalus" is the characteristic larval form of fishes of the orders Anguilliformes, Elopiformes, and Notacanthiformes) spawned in the southwestern Sargasso Sea in the winter of year t will be carried downstream in the Gulf Stream and will eventually detrain from the stream to invade the North American coast and the Gulf of St. Lawrence at the glass eel stage (immediately after metamorphosis) during the spring and summer of year t + 1 (Schmidt 1925; McCleave 1987).

From catch per unit effort data in a small stream entering the northern Gulf of St. Lawrence, Dutil et al. (1989) recorded a >90% decrease in glass eels between 1981 and 1982. The comparable proportionate decrease at the Saunders Dam ladder occurred in 1986 (Fig. 2), suggesting that the 750-km upstream migration from the Gulf of St. Lawrence would last on average 4 yr, bringing the eel there in year t + 5. Protracted upstream migrations of American eels have also been observed in tributaries of the Gulf of St. Lawrence, where some juvenile eels may not reach falls only 4 km upstream from the tributary's estuary before their third year of freshwater residence (Dutil et al. 1989).

Recaptures of 34 migrating adult eels (so-called silver eels) in the St. Lawrence River, originally tagged as yellow eels at the ladder, showed that on average, metamorphosis of yellow eels into silver eels occurred 10.3 yr (range = 6–15) after they had climbed the ladder (Fig. 3) (M.J. Eckersley, unpublished data; A. Michaud, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation, 1665 Boul. Hamel O., Québec, QC G1N 3Y7, unpublished data). Tagging may possibly have biased residence time in Lake Ontario, but this is unknown. The typical silver eel will therefore migrate down the St. Lawrence River in the fall of year t + 15 and spawn in the Sargasso Sea in the winter of year t + 16. As *A. rostrata* mature at greater ages and sizes in northern than in southern stocks (Helfman et al. 1987), SLR eels, at the north of the species' range, probably have a relatively long life span.

The SLR eel stock is composed exclusively of females; males are found primarily in the southern part of the species' range (Helfman et al. 1987). Desjardins et al. (1983b) and Dutil et al. (1987) found only females in a total of 763 SLR eels that were sexed histologically. Of the 315 SLR eels externally examined by Vladykov (1966), there was only one male. C.M. Couillard et al. (unpublished data) made histological and external sexual determinations of 105 and 568 SLR eels, respectively; only females were found. Jessop (1987) found only 1% male eels in Nova Scotia streams. However, male eels constituted 90% of a stock from a Rhode Island coastal stream (Winn et al. 1975).

An experimental trap net fishery for the purpose of providing live fish specimens to the Québec City Aquarium provides the only abundance index of the SLR silver eel migration. This fishery has been conducted in a standard fashion, in the same location (St. Lawrence River in Québec City) during the same period each year since 1971 (Robitaille et al. 1987). Before 1980, total annual silver eels catches fluctuated from 237 to 582 eels; from 1980 to 1986, catches were stable, slightly above 300 eels, but since 1987 the annual catches have been slightly below 300 eels (Fig. 4) (Robitaille et al. 1987; S. Pilote, Aquarium de Québec, 1675 des Hôtels, Sainte-Foy, QC G1W 4S3, personal communication).

Evidence of a Recruitment Decline

Annual numbers of juvenile eels climbing the Saunders Dam eel ladder decreased from a peak of 1 293 570 in 1983 to 935 170 in 1985 (the last year before the decline started) and went as low as 11 533 eels in 1992 (Fig. 2). This 81-fold or 99% decline appears to represent a real recruitment decline to Lake Ontario, as the Saunders Dam ladder is very efficient for indexing relative abundance of migrating eels (Casselman et al. 1990). According to Liew (1982), the ladder is the only passage for upstream migrant eels except for the Seaway

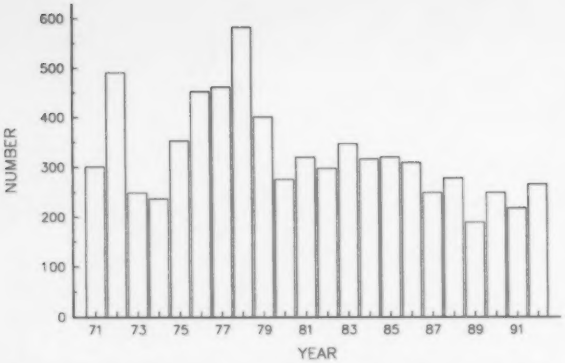

FIG. 4. Number of silver eels caught each year in the Québec City Aquarium standard experimental fishery (drawn from Robitaille et al. 1987; S. Pilote, Aquarium de Québec, 1675 des Hôtels, Sainte-Foy, QC G1W 4S3, personal communication).

locks. The eel ladder, 160 km downstream of Lake Ontario (Fig. 1), was built in 1974 and operated until 1979 (Eckersley 1982). A new ladder was built during the winter of 1981 for safety reasons. Before 1987, three 15-min daytime counts of eels were made each day and extrapolated to whole days using information from 24-h counts. In 1987, a Smith-Root model SR-1600 fish counter was installed to record total daily eel counts automatically. The fish counter was calibrated by visual observations. The ladder typically operates throughout the eel run (June–September or October), which lasts from 75 to 125 d. The mean annual length of yellow eels sampled at the dam increased from 35–40 cm in the early 1980's to 40–45 cm in the late 1980's/early 1990's (Liew 1982; Hartley 1992).

Standard electrofishing surveys carried out each year to assess Atlantic salmon (*Salmo salar*) parr abundance in tributaries to the Gulf of St. Lawrence provide evidence that the eel recruitment decline is not restricted to the upper St. Lawrence River, but is in fact occurring in other tributaries to the Gulf of St. Lawrence (Fig. 5). Of 20 Québec rivers in which such surveys are conducted, only two had a mean annual eel bycatch considered sufficiently large (>30 eels) to allow meaningful interannual comparisons. These electrofishing surveys, carried out in the Matapédia River between 1982 and 1987 and the Bec-Scie River between 1988 and 1992 (Fig. 1), indicate that yellow eel densities have declined significantly over the past decade (Kruskal–Wallis tests, $P < 0.05$). Such declines are indicative of a lower recruitment to these rivers. The mean annual number of 100-m^2 stations electrofished each year in the Matapédia and Bec-Scie rivers was 15 and 17, respectively, yielding a total of 40 and 158 eels per year on average, respectively.

As mentioned earlier, a study by Dutil et al. (1989) also pointed to an eel recruitment decline in the Gulf of St. Lawrence between 1981 and 1985. They recorded a <90% decrease in glass eel abundance in Petite Trinité River between 1981 and 1982 without a subsequent recovery. Moreover, standard surveys of juvenile eels conducted at waterfalls of the same river show an 80% decline between 1983 and 1984 (Dutil et al. 1989).

The recruitment decline to the Gulf of St. Lawrence could be as pronounced as in the upper St. Lawrence. The former index is a measure of "population" density, while the latter

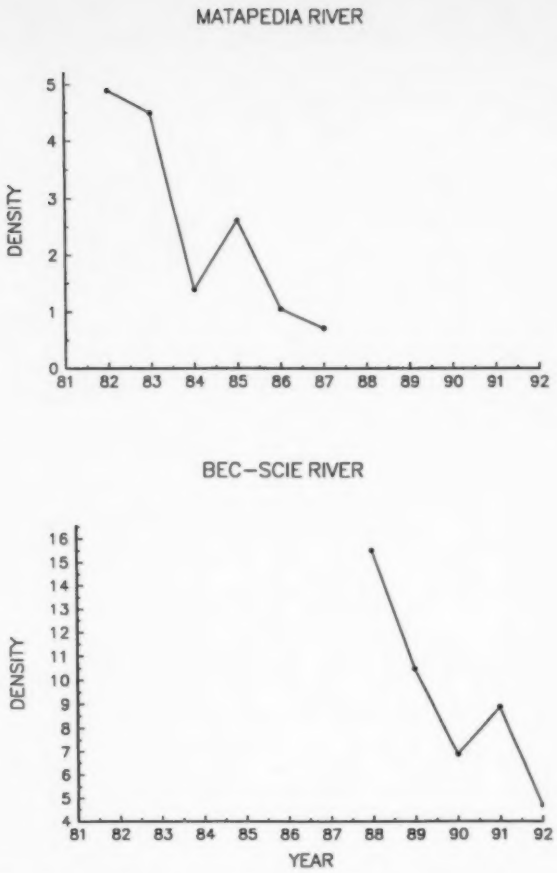

FIG. 5. Annual densitites of yellow eels in two rivers flowing to the Gulf of St. Lawrence, as determined by electrofishing surveys. Densities are expressed as mean number of eels caught per 100-m^2 fishing stations.

is a measure of recruitment. A glass eel recruitment decline of two orders of magnitude in Gulf tributaries could conceivably produce density declines similar to the ones observed in this study. Density-dependent effects could also amplify the magnitude of a recruitment decline at the head of a watershed, as lower population pressure may result in a decrease in the proportion of eels that reach the head of a drainage basin, as suggested by Moriarty (1986).

Assessment of Toxicity from Chemical Contamination

Evidence of Chemical Contamination

Large-scale surveys of chemical contamination of silver eels in the St. Lawrence River by mercury, polychlorinated biphenyls (PCB), pesticides, and mirex in 1982 and 1990 were compared (Desjardins et al. 1983a, 1983b; Dutil et al. 1985; Castonguay et al. 1989; Hodson et al. 1992, 1994). Levels of PCB and mirex of SLR silver eels declined by 68 and 56%, respectively. Geometric mean levels for PCB and mirex of 4.54 and 0.07 $\mu g \cdot g^{-1}$, respectively, for eels caught in the St. Lawrence estuary in 1982 were calculated from

the data of Desjardins et al. (1983a, 1983b) and were considerably higher than those for PCB and mirex of 1.12 and 0.025 $\mu g \cdot g^{-1}$ found in 1990 (Hodson et al. 1994). SLR eels in 1990 were much less contaminated with PCB than European eels from the Rhine River (Duursma et al. 1991), based on a comparison of PCB congener No. 138, the only congener in common between the two studies.

In Lake Ontario, levels of PCB, mirex, and pesticides in lake trout (*Salvelinus namaycush*) declined dramatically during the 1970's, but have shown little change since 1980 (EC/DFO/HWC 1991). The average 10.3-yr period of residence by eels in Lake Ontario may account for the difference between 1982 and 1990, since those sampled in 1982 would represent contamination during the 1970's, when chemical concentrations were higher in Lake Ontario.

Despite the decrease in chemical contamination of eels that occurred in the 1980's, SLR silver eels in 1990 were more than two orders of magnitude more contaminated than a reference stock from Rivière aux Pins, an uncontaminated tributary of the St. Lawrence estuary (Fig. 1) (Hodson et al. 1994). Concentrations were highest in the gonads and least in the head, and the differences among tissues followed closely the distribution of lipids.

Evidence of Toxicity

Summer mortalities of silver eels in the freshwater part of the St. Lawrence peaked in the early 1970's (Dutil 1984). Dutil et al. (1987) found that moribund eels had damaged gills and died from ion loss in freshwater. They argued that ion loss was produced by an increaced permeability of damaged branchial membranes caused by migration through highly polluted waters of the St. Lawrence. Diseased and healthy eels had similar pesticide and heavy metal loads, suggesting that mortalities were not due to chronic toxicity from accumulated contaminants but to acute toxicity from environmental contaminant levels. Furthermore, eel mortalities were not associated with bacterial pathogens (Dutil and Lallier 1984).

In 1990, 15 of 105 SLR eels sampled randomly had precancerous hepatic lesions as compared with none among 15 silver eels from the Rivière aux Pins reference group (C.M. Couillard et al., unpublished data). Furthermore, 7 of 673 SLR eels had spinal deformities as compared with none among 93 eels from Rivière aux Pins. Radiographs demonstrated that 18% of 427 SLR silver eels had vertebral fusions compared with 6% of 93 silver eels from the reference group.

Because fusions are probably healed injuries, SLR eels must face a more stressful environment than the reference group, due either to pollution or to physical damage. Certainly, chronic exposure of fish to either heavy metals or chlorinated organic compounds can lead to a variety of spinal deformities (Bengtsson and Larsson 1986; Bengtsson et al. 1988). However, given the number of canals and hydroelectric dams on the St. Lawrence and its tributaries, the spinal injuries may have been caused by mechanical factors such as water shear and turbines.

C.M. Couillard et al. (unpublished data) detected no histological abnormalities in SLR silver eel ovaries. However, reproductive failure might occur at a later stage of maturation, when eels are no longer available to sampling gears. Survivorship of larvae might also be compromised by contamination, since levels of total DDT of eel gonads sampled in 1990 (geometric mean of 0.37 $\mu g \cdot g^{-1}$; Hodson et al. 1992) were similar to levels associated with decreased viability of eggs and larvae in salmonid fishes (reviewed by Giesy et al. 1986). Cases of reproductive failure induced by chemical contamination have been reported for salmonid fishes from the Great Lakes (reviewed by Evans et al. 1990), and lifetime exposure to chlorinated organic compounds can also have sublethal effects on reproduction, as found in lake trout (Mac and Edsall 1991).

Toxicity from chemical contamination might be a contributing factor to the recruitment decline. There is strong circumstantial evidence of toxic chemical effects on Great Lakes fishes (Hodson 1987). However, Lake Ontario and the St. Lawrence River have been contaminated for a long time and lethal toxicity has been known to occur in SLR eels for over 30 yr (Dutil 1984). If toxicity were a major factor affecting eel recruitment, it seems that its effects would have been felt earlier. Certainly, acute mortalities of silver eels in freshwater (Dutil 1984; Dutil et al. 1987) reduce the numbers reaching the spawning grounds, but glass eel recruitment started declining only in 1982 (see Dutil et al. 1989), about 20 yr after silver eel mortalities started in the St. Lawrence and 10 yr after they peaked (Dutil 1984). If these mortalities had had a strong impact on the population, the recruitment decline would have been felt earlier.

The condition factor (defined as $100 \cdot W \cdot TL^{-3}$ where W = weight (grams) and TL = total length (centimetres)) of SLR silver eels was significantly higher in 1982 (0.213 ± 0.032) (mean ± SD) (calculated from Desjardins et al. 1983b) than in 1990 (0.204 ± 0.030) (calculated from Hodson et al. 1992) (Kruskal–Wallis test, $P < 0.0001$). This decrease over time, albeit small, suggests that silver eels faced a more stressful environment in 1990 than in 1982. The lipid reserved of prespawning eels en route to the Sargasso Sea may have decreased slightly during the 1980's; hence, SLR eels may now be less likely to complete their spawning migration and reproduce successfully despite lower contaminant levels. However, it seems unlikely that a slightly reduced condition of SLR silver eels could provide an 81-fold recruitment decline in the upper St. Lawrence River.

Assessment of Habitat Modifications

Anthropogenic habitat modifications in the Lake Ontario/St. Lawrence River ecosystem (see Whillans 1982; Robitaille et al. 1988; Hartig et al. 1991; Marquis et al. 1991) occurred mostly before the 1960's whereas the eel recruitment decline started only in 1982 and 1986 in the Gulf of St. Lawrence and Cornwall, respectively. Robitaille et al. (1988) and Marquis et al. (1991) identified the mid-1950's as the period when the most important permanent disturbances occurred in the St. Lawrence River. These disturbances (channel dredging, river flow change, yearly maintenance operations, mechanical effects of ships in transit) were associated with the construction and the operation of the St. Lawrence Seaway, which allows ships to travel upstream to the Great Lakes through a complex system of ship locks (Robitaille et al. 1988). Several hydroelectric dams, all built before 1965, operate in the St. Lawrence River. No changes in dam operation occurred during the 1980's (R. Verdon, Hydro-Québec, 1010 Ste-Catherine E., Montréal, QC H2L 2G3, personal communication).

Until 1984, the sewage of 92% of the human population

TABLE 1. American eel commercial landings (metric tons) in North America between 1975 and 1991. See text for sources of statistics.

Year	St. Lawrence River	SLR stock[a]	Atlantic Canada	United States	Total for species	SLR as % of total
1975	528.8	706.9	598.4	71.0	1376.3	51.4
1976	422.6	585.2	492.0	86.7	1163.9	50.3
1977	504.8	686.7	213.6	80.2	980.5	70.0
1978	518.7	760.6	207.2	117.0	1084.8	70.1
1979	498.7	730.9	225.8	103.6	1060.3	68.9
1980	591.3	786.8	347.1	86.3	1220.2	64.5
1981	593.1	745.3	474.6	78.1	1298.0	57.4
1982	322.0	390.3	356.2	130.5	877.0	44.5
1983	345.8	412.7	516.6	142.2	1071.5	38.5
1984	384.7	501.0	307.0	244.7	1052.7	47.6
1985	342.0[b]	446.6	428.0	167.2	1041.8	42.9
1986	504.5	621.5	531.5	207.4	1360.4	45.7
1987	428.9	532.7	398.8	116.5	1048.0	50.8
1988	431.2	532.9	532.0	103.4	1168.3	45.6
1989	433.4	556.0	469.0	394.9	1419.9	39.2
1990	475.1	591.7	455.0	573.0	1619.7	36.5
1991	397.0	512.2	495.8	673.9	1681.9	30.5

[a]Subtracting the St. Lawrence catch from the SLR catch gives the Lake Ontario catch.

[b]Incomplete landings because the freshwater part of the St. Lawrence was not available for that year.

inhabiting the shores of the St. Lawrence was untreated and discharged directly to the river (Anonymous 1991). Since 1987 the waste of 1.8 million people of the Montréal urban community has been consolidated from multiple untreated discharges to one treated chlorinated discharge at the east end of the Island of Montréal. As a result, 40% of the human population inhabiting the shores of the St. Lawrence now have their sewage treated (Anonymous 1991).

The Great Lakes have a history of changes due to (1) overfishing of indigenous piscivorous fish species that occurred before the 1940's (e.g., Christie 1973), (2) invasion of exotic species (e.g., Christie 1973), and (3) eutrophication (e.g., Hartig et al. 1991) that occurred before a phosphorous management strategy was put in place in 1972.

Habitat modifications, and especially construction of the St. Lawrence Seaway in the 1950's, have been proposed as the main factor involved in the decline of several anadromous populations (Atlantic sturgeon (*Acipenser oxyrhynchus*), striped bass (*Morone saxatilis*), Atlantic tomcod (*Microgadus tomcod*), rainbow smelt (*Osmerus mordax*), American shad (*Alosa sapidissima*), and lake whitefish (*Coregonus clupeaformis*)) which had been abundant in the St. Lawrence River before the 1960's (Robitaille et al. 1988). The decline of these populations occurred mostly before 1965, that is, shortly after the major habitat perturbations related to the construction of the seaway. Moreover, construction of hydroelectric dams on the St. Lawrence may also have played a major role in the decline of anadromous populations. In some cases, they left only ship locks as a way for fish to move upstream. The close temporal correspondence between these perturbations (seaway and dams) and the decline of anadromous populations provides strong circumstantial evidence for a cause–effect relationship.

In contrast with anadromous populations, the lack of temporal correspondence between permanent habitat modifications in the 1950's and the start of the eel recruitment decline in the 1980's argues against their role in the decline. If the effect of habitat modifications in the St. Lawrence on eel recruitment were important, they should have had an impact much before 1982 because, by that time, they had been occurring for 25–30 yr. However, because of their strikingly different life histories, catadromous populations are likely to respond more slowly to anthropogenic perturbations compared with anadromous populations. The eel life style is substantially longer than that of anadromous species (except sturgeon), and in contrast with anadromous populations, eel spawning sites were not affected by habitat modifications. Also, because of their ability to bypass obstacles (e.g., Dutil et al. 1989), eels may be more resilient to dams and other man-made obstacles than anadromous fishes. Toxicity and habitat modifications in the St. Lawrence and elsewhere could still have played a key role in the recruitment decline, but their effects would have had to have been delayed through some unknown compensatory population process.

Assessment of Commercial Fishing

Trends in commercial eel landings in Canada and the United States were compared with the timing of the decline to assess possible impacts of fishing mortality (Table 1), although the absence of fishing effort information greatly limits such an assessment. The Lake Ontario fishery (sources: Blake 1982; Kolenosky and Hendry 1982; P. Smith, Ontario Ministry of Natural Resources, P.O. Box 605, Oxford Ave., Brockville, ON K6V 5Y8, personal communication) is localized on the Canadian side of the lake. Before 1983, less than 50 t (included in Table 1) was also reported on the U.S. side of the lake, but that fishery was closed permanently in September 1982 due to high levels of mirex in eels. The St. Lawrence River fishery (before 1986: Robitaille et al. 1988; Bérubé 1990; after 1985: Johnson 1990; G. Johnson (Ministère de l'Agriculture, des Pêcheries et de

l'Alimentation, 200-A Chemin Ste-Foy, Québec, QC, G1R 4X6, personal communication)) is localized mostly in the St. Lawrence estuary and Lake St. Pierre, an enlargement of the St. Lawrence River (Fig. 1). The Atlantic Canada fishery (sources: LeBlanc and Chaput 1991; J. Kane, Department of Fisheries and Oceans, P.O. Box 550, Halifax, NS B3J 2S7, personal communication) includes landings from the Gulf of St. Lawrence and the Atlantic coast of Nova Scotia. The source of U.S. statistics is the National Marine Fisheries Service, Statistics Division, Woods Hole, MA.

The total commercial catch of American eels in North America between 1975 and 1991 has varied between 877 t in 1982 and 1682 t in 1991, with the SLR stock representing between 31 and 70% of the total (Table 1). Before 1989, there was no evidence of upward or downward trends in total annual catches. Since 1989 the total catch has increased because of larger catches in the United States (primarily in Delaware, Maryland, and Virginia). Although the total North American catch did not exhibit trends before 1989, there was a decrease in landings of the SLR stock in the 1980's which coincides with the timing of the recruitment decline. The mean annual catches of SLR eels from 1975 to 1981 and from 1984 to 1991 were 715 and 537 t, respectively. The low 1982 and 1983 catches of SLR eels were due to a temporary loss of markets for fishermen caused by high levels of chemical contamination by mirex.

Total annual commercial catches of SLR eels (Table 1, excluding 1982 and 1983 for reasons presented above) were marginally significantly correlated with catches of the Québec City experimental fishery (Pearson's $r = 0.49$; $P = 0.06$) but the St. Lawrence River catches alone (excluding Lake Ontario) were not ($P > 0.2$). This marginally significant correlation indicates that SLR catches may fluctuate according to abundance, implying that the level of fishing effort and the exploitation rate may not have varied much from year to year and that commercial fishing may not have been a major factor contributing to the recruitment decline of the eel. Considering the stable North American landings between 1975 and 1988, the overall commercial exploitation rate on the population (species) may have remained fairly stable during that period. However, the steep catch increase observed in the United States since 1989 indicates that the exploitation rate has probably recently increased. This may have had an effect on recruitment, but to an unknown degree.

SLR annual eel catches were on average 25% smaller during the 1984–91 period than during the 1975–81 period, but they have remained fairly stable since 1984. The exact year in which the stepwise catch decline between the late 1970's and the 1980's occurred is unclear because of the mirex crisis described above. The Québec City aquarium standard fishery has declined slightly since 1987 (Fig. 4). Assuming that the two above declines reflect a decline in SLR silver eel abundance in the 1980's, there might be a cause–effect relationship with the lower juvenile eel recruitment to the Saunders Dam ladder if, as argued before, the SLR stock represents a major source of females for the species. However, it seems unlikely that a slightly reduced abundance of SLR silver eels could provoke an 81-fold recruitment decline in Cornwall.

The major weakness of the above considerations is the absence of fishing effort information. A recent attempt by J.A. Robitaille (Bureau d'écologie appliquée, 4950 Boul Rive Sud, bureau 104, Lévis, QC G6V 4Z6, personal communication) to analyze this fishery further suggested important changes in fishing effort and catch in specific zones of the St. Lawrence River. The overall trend is for a decrease in effort and catch in the freshwater portion and an increased effort and catch in the saltwater fishery. Is it possible that changes of fishing effort have masked a long-term decline in escapement of the SLR stock?

Assessment of Oceanic Changes

Results of a recent study that quantified the interannual variability of Gulf Stream transport (Greatbatch et al. 1991) were interpreted in the context of recruitment variability of eels. These authors presented calculations of the circulation in the North Atlantic which indicate that the Gulf Stream was 30 Sverdrups (30×10^6 $m^3 \cdot s^{-1}$) weaker in the early 1970's than in the late 1950's. This represents a decrease of about one third when compared with Richardson's (1985) estimate of 93 Sverdrups for the long-term average transport of the Gulf Stream through 55°W. Such variability in transport most likely affects *Anguilla* recruitment because their larval migration towards the continents is achieved by drift (Schmidt 1925; Power and McCleave 1983; Helfman et al. 1987; McCleave 1987). Unfortunately, no Gulf Stream transport calculations are yet available for the 1980's, which could be directly compared with the recruitment decline of SLR eel stock.

It seems improbable that a Gulf Stream weakening in the early 1980's similar to the one between 1970 and 1974 (i.e., a 33% decrease) (Greatbatch et al. 1991) could explain the dramatic eel recruitment decline observed in the 1980's. The low Gulf Stream transport of the 1970–74 period nevertheless produced large numbers of juvenile eels at the Saunders Dam ladder between 1975 and 1979 (Fig. 2). Indeed, eel larvae produced between 1970 and 1974 reached the ladder between 1975 and 1979, as we estimated that the juvenile eels reach the ladder on average 5 yr after they are transported down the Gulf Stream as larvae. The effects that a larger weakening of the Gulf Stream in the 1980's, compared with that of 1970–74, would have on recruitment are unknown.

McCleave (1987) proposed the following hypothetical scenario of continental invation of eel larvae, which is based on existing physical processes and known elements of the behavioral repertoire of the eel. Vertically migrating leptocephali are first advected downstream by the Gulf Stream and then detrain passively from the stream through physical processes of diffusion, eddies, and intrusions of Sargasso Sea water onto slope water. Leptocephali that are ontogenetically ready then metamorphose into glass eels when they encounter the sea bottom. At that time the animals would switch from a diel vertical migration rhythm to a tidal rhythm and use selective tidal stream transport (e.g., McCleave and Kleckner 1982) to reach the continent. The effects of the cooling event and the related oceanographic changes that are currently taking place in the Northwest Atlantic (e.g., Drinkwater 1992) on continental invasion of glass eels are also unknown.

Conclusions and Recommendations

Because of panmixia, recruitment variability of the American eel must be interpreted at the level of the whole

species. For a high prespawning mortality of SLR eels to affect subsequent recruitment to the St. Lawrence, the effect must be felt in the whole population. The SLR stock may conceivably be large enough to produce such an effect if a high mortality were to occur, as it may represent a primary source of females for the population. The St. Lawrence drainage basin in composed exclusively of females and is also where the largest and most fecund females are found (Helfman et al. 1987). The importance of SLR eel production is further illustrated by the following statistic: the freshwater runoff of the St. Lawrence River (10 100 $m^3 \cdot s^{-1}$) represents approximately 19% of the total freshwater runoff in the species' range (53 000 $m^3 \cdot s^{-1}$) (Todd 1970; Anonymous 1978), as defined by Scott and Crossman (1973) (excluding the small part of the range south of the United States).

Alternatively, a decrease in SLR silver eel production could have had little effect on subsequent recruitment, while the two events could remain fairly well synchronized because the timing of man-made perturbations (chemical contamination and habitat modifications) related to the acceleration of industralization that followed the Second World War was similar throughout the species' range. Hence, man-made adverse effects may have acted in a fairly synchronous manner. However, the latitudinal cline in the length of the eel life cycle (Helfman et al. 1987) complicates the interpretation of any recruitment decline in this species. The above considerations exemplify the difficulties of determining causes of population fluctuations in panmictic species. The eel life history is too complex to permit an unequivocal determination of causes of the recruitment decline.

A further complication concerns possible synergism of causes. For example, the recruitment decline could be primarily due to an oceanic factor but could be amplified by chemical contamination which may reduce escapement of adults to the spawning site. Or it might be that fishing did not have deleterious effects before the major habitat modifications took place, but that it might have such effects now because habitat modifications may have reduced escapement substantially.

Because of these complications, it may not be surprising that we are forced to conclude that, by and large, we do not really know what caused the pronounced recruitment decline observed in this species. The 25- to 30-yr delay between anthropogenic environmental perturbations (toxicity and habitat modifications) and the eel recruitment decline in the St. Lawrence argues against these perturbations as primary causes of the decline. However, we cannot rule out that their impact may have been significant but delayed through some unknown compensatory population process. Commercial fishing may have possibly played a role in the decline, but there is little evidence that it did. Alternatively, the decline could primarily be due to a natural phenomenon, namely oceanic changes.

In light of the current status of eel stocks in the St. Lawrence, research is obviously needed to document future trends in abundance and to study causes of the decline. A workshop on this problem was held in Québec City in March 1993 (see AFS Atlantic International Chapter August 1993 Newsletter for details) at the end of which several research recommendations were made by participants. The following recommendations are partly drawn from discussions at this workshop.

First, attempts will be made to construct additional eel abundance series from other electrofishing surveys carried out in other parts of the species' range. Such surveys would allow us to test the hypothesis that the recruitment decline is due to oceanic changes and hence would not be species wide. More specifically, we hypothesize that due to a weak, slow Gulf Stream (weaker than between 1970 and 1974), larvae may have metamorphosed or missed the optimum period for metamorphosis and were lost to the population when they reached the position of the stream where lateral transport would have placed them in the Gulf of St. Lawrence. An alternative oceanic hypothesis is that one or several of the physical processes that carry glass eels to the continent (see McCleave 1987) may have been perturbed by the recent cooling event and the related oceanographic changes that are taking place in the Northwest Atlantic (Drinkwater 1992). They could act in such a way as to reduce the probability that glass eels will reach the continent at Gulf of St. Lawrence latitudes. However, if we establish that the recruitment decline is widespread, consideration should be given to adding the eel to the endangered species list and organizing a Canada–U.S. workshop on the subject.

A second research recommendation would be to conduct a tagging program of SLR silver eels to estimate the exploitation rate by the commercial fishery. Such a study may allow us to test the hypothesis that the St. Lawrence commercial fishery is not responsible for the current decline. Also, reestablishing an eel survey that was formerly carried out in the Petite Trinité River (see Dutil et al. 1989) will allow a comparison of current abundances with those measured in the early 1980's. Furthermore, developing a catch per unit of effort series would make better use of available commercial fishery data, while pursuing studies on toxic effects of contaminants on gills and gonad development would permit a better assessment of the role of chemical toxicity. Lastly, two management recommendations were put forward at the workshop: to halt the issue of new commercial eel fishing licenses in the St. Lawrence and to stress to hydroelectric companies the importance of building eel ladders to allow safe passage of upstream and downstream migrants.

As a final note, it is important to reemphasize that, because of panmixia, the eel recruitment decline observed in the St. Lawrence River and Gulf could be species wide. Even though it might not currently be species wide, a continuing decline would eventually affect the entire species because of the relative importance of the SLR eel stock.

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